METABOLISM OF ALDICARB, ALDICARB SULFOXIDE AND ALDICARB SULFONE IN POTATO PLANTS AND IN PLANT CALLUS TISSUE CULTURES OF POTATO TUBER AND CITRON FRUIT

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1986

To Jessica, Julia and Joel

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Willis B. Wheeler for serving as chairman of my doctoral research committee. His efforts through encouragement, guidance and support provided the necessary atmosphere and environment for me to successfully complete this work.

I would also like to thank and acknowledge the other members of my supervisory committee. Dr. N. P. Thompson provided valuable encouragement and advice. Dr. D. A. Carlson, Dr. H. A. Moye and Dr. M. D. Corbett all contributed valuable advice, helpful discussions and constructive criticism.

I thank Ms. Philippa Angelides for typing this dissertation and Ms. Susan Scherer for drafting the figures. I am grateful to all of my colleagues at the Pesticide Research Laboratory for their friendship.

Finally, I thank my wife, Jan, and our three children, Jessica, Julia and Joel, for their patience and support.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

METABOLISM OF ALDICARB, ALDICARB SULFOXIDE AND ALDICARB SULFONE IN POTATO PLANTS AND IN PLANT CALLUS TISSUE CULTURES OF POTATO TUBER AND CITRON FRUIT

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May, 1986

Chairman: Willis B. Wheeler
Major Department: Food Science and Human Nutrition

The metabolism of ^{14}C-aldicarb [2-methyl-2-(methylthio) propionaldehyde-0-(methylcarbamoyl) oxime], ^{14}C-aldicarb sulfoxide [2-methyl-2-(methylsulfinyl) propionaldehyde-0-(methylcarbamoyl) oxime] and ^{14}C-aldicarb sulfone [2-methyl-2-(methylsulfonyl) propionaldehyde-0-(methylcarbamoyl) oxime] was determined in *in vivo* potato plants and in *in vitro* callus tissue cultures derived from potato tuber (*Solanum tuberosum*) and undeveloped seed ovules from the fruit of a citron tree (*Citrus medica*). Potato plants were treated with the three compounds in separate experiments and were harvested two, six and 10 weeks post application. The foliage, stem, seed potato,

tuber and root of the plants were analyzed individually. Aldicarb was degraded rapidly and was not detected in any plant part at 10 weeks post application. Aldicarb sulfoxide and aldicarb sulfone were extensively degraded but 6.2 ppm and 7.5 ppm was still present at 10 weeks, respectively. Plant foliage contained the highest percentage of plant ^{14}C residues (> 90.0%) for all three compounds and percentages detected in the other plant parts ranged between 0.5 and 5.0%. The data indicated that sulfoxidation of aldicarb to aldicarb sulfoxide was a major reaction pathway and further oxidation to aldicarb sulfone was not. Aldicarb sulfoxide was decarbamylated to varying degrees in different plant parts to yield oxime, nitrile, alcohol, acid and amide metabolites. Half lives of the parent compounds in the whole plant were less than two weeks for aldicarb, 28 days for sulfoxide and 25 days for sulfone.

Treated callus tissues were analyzed for up to 27 days post application. The half lives of aldicarb, aldicarb sulfoxide and aldicarb sulfone in the tuber callus were 6.9, 28.0 and 11.0 days, respectively, and in citron callus, 2.8, 68 and 61 days, respectively. Tuber callus tissue produced higher percentages of hydrolysis products and water soluble ^{14}C material than the citron callus tissue. Formation of aldicarb sulfone was not significant. Qualitatively, the metabolic products found by both callus tissue systems and the in vivo potato plants were similar.

CHAPTER I

INTRODUCTION

Background

Aldicarb, 2 - methyl - 2 (methyl thio) propionaldehyde 0-(methylcarbamoyl)-oxime, is a carbamoyl oxime carbamate insecticide produced and marketed by Union Carbide Agricultural Products Company, Inc. It is the active ingredient in the granular formulation Temik(R), the registered trademark of Union Carbide. Aldicarb is used to control certain insects (aphids, thrips, leaf hoppers and beetles), mites and nematodes and is registered for use on cotton, peanuts, potatoes, sugar cane, sugar beets, sweet potatoes, oranges, beans, soy beans and pecans. The formulation exerts systemic action and is generally applied to the soil at planting and/or post emergence (Union Carbide form no. AG820037.5M-11/81-TCG). The active ingredient is rapidly absorbed by roots and translocated throughout all parts of the plant. Control of pests often lasts longer than six weeks, but varies with growth conditions, rate of use and pests. Increased crop yield has also been reported to be a result of Temik(R) use. The

mechanism of this effect is unclear and could be due to reduced stress on the plant from pests or to growth stimulation.

Aldicarb was developed during the mid 1960s by Union Carbide when its synthesis (Bartley et al., 1966, and Payne et al., 1966) and insecticidal properties (Payne et al., 1966) were reported. Consequently, an abundance of literature exists describing the behavior of aldicarb in the environment and in various biological systems. Aspects of aldicarb behavior which will be considered in this review include: occurrence as residues in plants and soil; general chemical and physical properties, biological metabolism in plants, fungi, rats and plant microsomes, biological mode of action, and miscellaneous analytical techniques.

Recent concern about aldicarb developed from the contamination of groundwater on Long Island, New York, and in Florida after usage in these areas for agricultural purposes. Considerable data has been generated regarding the dynamics of residue distribution and the ultimate environmental fate. Although aldicarb is used in many other locations throughout the United States, the situations in New York and Florida have stimulated much research on the environmental fate and transport of this compound and its toxic metabolites.

Temik aldicarb pesticide was used in Suffolk County, Long Island, from 1975 to 1979 by potato farmers for insect and nematode control. In late 1979 and 1980, analyses showed that residues from Temik applications did reach drinking water supplies. This discovery led to the ban of Temik use on Long Island and initiation of studies to assess the extent of groundwater and drinking water contamination. Over 10,000 wells were sampled; approximately 15% contained detectable levels of aldicarb residues. The monitoring of wells during the period of 1980 to 1984 indicated a reduction in the average concentration of aldicarb residues. Twenty-seven wells had an average aldicarb "total toxic residue" (aldicarb, aldicarb sulfoxide, aldicarb sulfone (TTR) in 1980 of 47 ug/L and by 1983 this level had dropped to 27 ug/L. Over half the wells had aldicarb TTR concentrations exceeding 7 ug/L. Laboratory studies indicate a half-life of 2-3 years for aldicarb TTR in Long Island groundwater. Research at Cornell University is continuing to assess the long-term fate of aldicarb (Porter et al., 1984).

The Florida situation developed in 1983 when there arose concern about possible contamination of orange juice and citrus products by aldicarb TTR. Aldicarb was used extensively in Florida as a systemic insecticide by the citrus and potato industries to control nematodes and other pests. A state-wide task force was formed to assess the

extent of citrus crop and potato crop contamination, and the behavior of aldicarb in citrus groves and potato production areas. Preliminary results indicated that aldicarb TTR were well below the tolerances in both citrus and potatoes. Laboratory and field studies showed, however, that the half-life of aldicarb TTR appeared to be longer than had been previously reported.

Statement of Purpose

The research results to be reported here were initiated, in part, to address questions concerning the metabolism of aldicarb and its toxic metabolites, aldicarb sulfoxide and aldicarb sulfone, in commodities which have economic importance in the State of Florida. One such commodity is the potato which is widely grown in North and North Central Florida. Florida potatoes are grown primarily for use in processed potato products such as potato chips. Aldicarb is used for insect and nematode control on the potato crop and is generally applied at a rate of 3.36 kg/ha as the Temik 10G granular formulation. Granules are incorporated into the soil at the time of planting or shortly thereafter. Through systemic action aldicarb and its residues become distributed throughout the plant as it matures. The objectives in this research component were to determine the uptake and distribution in potato plants of aldicarb, aldicarb sulfoxide and aldicarb

sulfone residues when applied individually and to determine the metabolic fate of these materials in the plant.

Another objective of this research was to investigate the metabolism of aldicarb, aldicarb sulfoxide and aldicarb sulfone in plant callus tissue culture systems. Some advantages of using tissue culture in metabolism experiments are that they can be performed under highly controlled conditions to overcome problems associated with in vivo experiments. Aseptic conditions isolate plant cell metabolism and eliminate microbial metabolism. Cultures can be grown in the dark to reduce photochemical reactions. This allows a more accurate evaluation of cellular metabolism. Two plant callus systems were chosen, potato tuber callus and citron callus. Potato tuber callus was developed from the same potato variety used in the in vivo plant experiment so comparisons could be made between the in vitro and in vivo results. The other plant callus system was citron callus derived from undeveloped seed ovules from citron fruit, a citrus species. Therefore, comparisons could be made between the two callus tissue culture systems.

CHAPTER II

REVIEW OF LITERATURE

Chemical and Physical Properties of Aldicarb

The physical and chemical properties of aldicarb have been studied to understand its behavior under various laboratory conditions. This type of information could be useful to make predictions or extrapolations to "real" situations or to at least give a basis for speculation.

The molecular weight of aldicarb is 190.25. Crystals from isopropyl ether have a melting point of 99-100°C. Its solubility at 25°C is: 0.6% (6000 ppm) in water; 35% in acetone; 15% in benzene; 5% in xylene; 30% in methylene chloride (Merck Index, 1976).

Photochemical reactions were investigated by Freeman and McCarthy (1984). They irradiated solutions of aldicarb in both water and acetonitrile and also in the presence of sensitizers (benzophenone, acetophenone and benzonitrile). Photolysis of aldicarb (0.1M) in water for 1 hour with a 450 watt Mercury lamp resulted in a product mixture containing α-methylacrylonitrile, dimethyl sulfoxide, 2-(methylthio)-2-methyl propane nitrile and many other

minor products. Irradiation in acetonitrile with the same light source at 250 nm yielded methyl amine, dimethyl sulfide, tetramethylsuccinonitrile and 1-(methylthio)-2,3-dicyano-2,3-dimethylbutane. Small amounts of N, N'-dimethylurea were also formed. The presence of sensitizers had no effect on the resultant products.

Lemley and Zhong (1983) investigated the kinetics of aqueous base and acid hydrolysis of aldicarb, aldicarb sulfoxide and aldicarb sulfone in abiotic distilled water. Base hydrolysis of ppm solutions of the three toxic metabolites exhibited first order behavior relative to the hydroxide ion. The sulfoxide and sulfone were hydrolyzed more rapidly than aldicarb. The presence of a neutral electrolyte reduced the base hydrolysis rate. Rate constants for acid hydrolysis were much lower than for base hydrolysis. Lemley and Zhong (1984) investigated further the hydrolysis of aldicarb, the sulfoxide and sulfone in chlorinated aqueous media and in well water samples. They found that base hydrolysis rates were slower in chlorinated water and in well water than rates measured in distilled water.

Bank and Tyrrell (1984) used pulse Fourier transform nuclear magnetic resonance to investigate the hydrolysis of aldicarb over the pH range 3.0 to 8.6 in deuterated phosphate buffers. Base catalyzed carbamate decomposition occurred above pH 7.0 forming, as primary products,

aldicarb oxime, dimethylurea, methylamine and carbon dioxide. Acid catalyzed reactions (pH < 5.0) formed principally aldicarb nitrile and methyl amine. Copper (II)-promoted hydrolysis of aldicarb in citrate buffers (pH 2.9 to 5.5) yielded products similar to those from simple acid catalyzed reactions principally aldicarb nitrile (82%) and aldicarb aldehyde (18%) (Bank and Tyrrell, 1985).

Degradation of aldicarb (Trehy et al., 1984 and Miles and Delfino, 1985) aldicarb sulfoxide and aldicarb sulfone (Miles and Delfino, 1985) was determined in Floridan groundwater microcosms. Trehy et al. (1984) found that aldicarb degraded rapidly to aldicarb nitrile in groundwater enriched with microorganisms (pH 6.8) or crushed limestone (pH 7.0 to 7.4) under anaerobic conditions. Miles and Delfino (1985) found base hydrolysis as a major reaction mechanism for degradation of aldicarb, aldicarb sulfoxide and aldicarb sulfone in Floridan groundwater. The primary products for the three compounds were the corresponding oximes and degradation rates decreased in the order sulfone > sulfoxide >> aldicarb. Addition of limestone resulted in a 4-5 times reduction in the hydrolysis rates.

Occurrence of Aldicarb Residues in Crops

The application of aldicarb as a granular formulation to plants grown under field conditions has yielded information about the occurrence of agricultural residues

and their dissipation. Important considerations include the uptake of residues from the soil, their distribution in the plant, and their reduction in concentration. In most of these studies the aldicarb TTR were determined by conversion of aldicarb and aldicarb sulfoxide to the sulfone. The TTR can be quantified by gas chromatography.

Awasthi et al. (1979) applied Temik 10G granules to potatoes at normal agricultural rates (1 to 5 kg active ingredient (a.i.)/ha). Residues were determined in 1) unpeeled, unwashed potatoes, 2) peeled, washed potatoes, 3) washed, boiled and peeled potatoes and 4) in the boiled water of potatoes, at the normal harvest period for potatoes, 90 days post planting, as well as after 30 days and 60 days of storage. From the 1, 2 and 3 kg/ha application, residues in unpeeled, unwashed potatoes at 90 days were 0.45, 0.62 and 0.95 ppm, respectively. For the 4 and 5 kg/ha applications TTR were 1.17 and 1.82 ppm, respectively. The EPA tolerance for aldicarb residues in potatoes is 1.00 ppm. Peeling reduced TTR by 63 to 65% and boiling followed by peeling removed 94-100% of the residue. Very small amounts were detectable in the boiled water (0.02 to 0.07 ppm). Storage of the 4 and 5 kg/ha treated potatoes for 30 and 60 days post harvest reduced TTR to below the 1.0 ppm tolerance. George et al. (1975) found that the number of days between planting and treatment had an effect on the level of residues in the

mature tuber at harvest. Residues were highest when treatment coincided with planting and were lower when planting was 35 or 57 days before treatment. The authors speculate that this was due to a more vigorous rate of growth of the plant in the early stages, and therefore, a more rapid uptake of aldicarb in early growth stages than in later ones.

Dejonckheere et al. (1982) studied the uptake of aldicarb by field grown sugar beet plants from three different soils, a sandy loam, silt loam and sandy clay loam. Residues were seven to ten times higher in leaves of plants grown in the sandy loam and silt loam soils than in the sandy clay loam. This leaf concentration correlated with the efficacy of aldicarb for aphid control which was poor for the sandy clay loam grown plants but was effective for plants grown in the other soils. Thus, on heavy clay loam soils it was necessary to apply higher rates of aldicarb to obtain the same level of aphid control as achieved with the other soil types.

Residues of aldicarb in a Pennsylvania scotch pine absorbed from soil treated at 0.23, 0.45 and 0.91 kg/12 trees were studied by Kearby et al. (1970). TTR residues had decreased to 0.0 ppm in the soil for the 0.23 and 0.45 kg application rates and less than 0.07 ppm for the 0.91 kg rate by 63 days post application. Aldicarb was readily absorbed and translocated by the trees. At 7 days post

application, residues were higher in the lower and upper branches of the tree than in the needles for all application rates. By 21 days more residue was in the upper needles and upper branches than in the lower parts of the plant demonstrating translocation to the terminal growing points.

Aldicarb residues in grapevine were determined by Hafez and Raski (1980). Residues were found in the leaves at 120 days post application as high as 1.4 and 8.89 ppm from application of 4.5 and 9.0 kg/ha, respectively. Residues had begun to decline by 180 days and continued to decline thereafter. No TTR was detected in the newly formed fruit, however, 0.03 and 0.05 ppm were detected in the mature grapes from the 4.5 and 9.0 kg/ha applications, respectively. Factors which contributed to the level of residue in the mature fruit were grape variety, time and rate of application, amount of rainfall and irrigation water and soil type.

In cauliflower, aldicarb was applied to soil at a rate of 1.5 kg/ha (Pareek et al. 1978). Aldicarb residues were 2.4 ppm in the leaves 15 days post application but were not detectable at 35 and 60 days. In the cauliflower core there was 1.97 ppm at 55 days and 0.45 ppm at 60 days. These levels exceeded the tolerance of 0.2 ppm for cauliflower.

Hendrickson and Meagher (1968) investigated aldicarb residues in component parts of citrus following soil applications of Temik 10 G at 0.11 kg/orange tree and 0.09 kg/grapefruit tree. The maximum concentration of TTR was found approximately 33 weeks after application in both the orange and grapefruit trees. More residue accumulated in the juice than in the peel of the fruit prior to full maturity. The order of concentration of TTR in the various parts of the tree were, from highest to lowest, roots, leaves and fruit.

Aldicarb 10G has been used by commercial growers for more than 10 years to control insect and mite pests on a wide range of greenhouse floricultural crops. Krueger et al. (1975) performed experiments to determine aldicarb residues in chrysanthemum foliage, buds and flowers resulting from soil incorporation or broadcast of granules over wet and dry foliage. The relationship of these two application methods to plant maturity at the time of application was also determined. The results showed that residues may not reach high enough levels to be toxic to certain insects and mites, regardless of application method. Plants treated when older had lower aldicarb residues than younger plants.

Krueger and Mason (1974) reported the effects of 23 plant growth regulators on the uptake and metabolism of aldicarb in soybeans under greenhouse conditions. The

plants were first treated with the growth regulator, then treated with aldicarb. None of the plant growth regulators had an effect on the uptake of aldicarb or its degradative metabolism.

Ferreira and Seiber (1981) studied the pathways of dissipation of aldicarb in rice grown hydroponically by contrasting the behavior of root applied and foliar applied materials. Volatilization of aldicarb from leaf surfaces occurred after rapid translocation to leaves, where residues moved through the leaf tip to the outside leaf surface by guttation. Approximately six percent of the total aldicarb residues were lost by this mechanism; fourteen percent was lost by root exudation.

Toxicological Mode of Action

Aldicarb is a carbamate insecticide, which, like organophosphates, exert their toxic action by inhibition of the enzyme acetylcholinesterase (AChE). The LD_{50} for aldicarb in mammals is less than 0.9 mg/kg. Thus, aldicarb is extremely toxic. Aldicarb is rapidly converted to the sulfoxide which is a more potent acetylcholinesterase inhibitor than aldicarb. Aldicarb sulfoxide toxicity, in most cases, is probably responsible for the acute toxicity of aldicarb exposure.

AChE is a hydrolase enzyme and one of two cholinesterases, the other being butyryl cholinesterase (BChE). Both kinds of esterases are common in vertebrates. AChE is

found in erythrocytes, nervous and muscle tissue while BChE is found in blood plasma, liver, nervous tissue and numerous other tissues. If AChE activity is blocked in neuromuscular junctions death usually results, however BChE can be inhibited with no apparent ill effects. There are dozens of known insect isozymes of AChE. The housefly, for example, has three in the head and four in the thorax. AChE inhibition of one isozyme in the thorax was responsible for death.

In mammalian species failure of the respiratory center of the brain is the most common cause of death from carbamate poisoning. This is due to nicotinic effects involving the neuromuscular junction which at first are excitatory, leading to muscle twitching, then inhibitory, leading to paralysis. Early symptoms involve parasympathetic stimulation such as lachrymation, hypotension, defecation and urination. Death results from asphyxiation due to respiratory failure.

There are several important characteristics of snyaptic transmission. In parasympathetic or cholinergic synapses there is a presynaptic and post synaptic membrane separated by approximately 100-200 Å. An action potential is built up by selective Na^+/K^+ concentration changes which induce release of acetylcholine from vesicles in the presynaptic membranes. The acetylcholine crosses the synaptic gap and is absorbed by the postsynaptic membrane

altering the permeability to ions of the membrane, thus allowing build up of a postsynaptic potential. AChE is necessary to degrade acetylcholine in order to facilitate transmission of a new impulse by restoring the resting action potential. AChE hydrolyzes acetylcholine to acetic acid and choline. If this hydrolysis is inhibited then acetylcholine accumulates at the postsynaptic membrane and permanent stimulation occurs resulting in the symptoms previously described.

The interaction of carbamates with AChE is similar to that of AChE and acetylcholine, however, it involves carbamylation of the serine hydroxyl group at the active site instead of acetylation. Where acetylated AChE is unstable and dissociates in approximately 0.1m sec the carbamylated enzyme is much more stable. The reaction is progressive and temperature dependent. It is: EOH + AX $\leftrightarrow$ EOH.AX $\rightarrow$ EOA $\rightarrow$ EOH + A^- + H^+ where EOH is the AChE, X is the leaving group and A is the carbamyl group. Spontaneous hydrolysis of the enzyme-carbamate complex can occur in the presence of H_2O. Aging does not occur with carbamates as with organophosphates.

The acute toxicity of aldicarb was determined by Landau and Tucker (1984) in two estuarine fish species, snook (Centropomus undecimalis) and juvenile minnows (Cyprinodon variegatus). Concern about aquatic species results from potential runoff of aldicarb into streams from

agricultural areas. The 48 hour LC_{50}'s for both snook and minnows was 0.1 mg/liter. A snook embryo/larva bioassay yielded a 36 hour LC_{50} of 0.04 mg/liter. Acetylcholinesterase activity has been detected on the surface of the embryos of the fish Oryzias latipes. The authors suggest that snook embryos may have similar characteristics and thus would be highly susceptible to aldicarb.

Kumar and Pant (1984) evaluated the histopathological impact of aldicarb on the fish Barbus conchonius. Pathological effects were observed on the gills, liver and kidneys. Fish were exposed to one-third of the 96 hour TLm value. After 2, 7 and 15 days of exposure fish were sacrificed and the gills, liver and kidney removed for histological analysis. The gills generally exhibited an over secretion of mucus with intense swelling and accumulation of blood. Liver histology revealed only minor alterations consisting of swollen capillaries and widespread vasodilation. Since hepatocytes remained uninjured during the bio-assay the authors suggested that there was either very little uptake of aldicarb by the liver or there was a very rapid metabolism within the liver to non-toxic compounds. The greatest damage was observed in the kidney. It suffered internal hemorrhage and congestion from ruptured capillaries and masses of blood cells packing the intertubular spaces. This was followed by degeneration of lymphoid tissue and necrosis of some

renal tubules. Despite these changes no mortality resulted from the treatment.

Mutagenicity studies have been done on aldicarb, aldicarb sulfoxide and aldicarb sulfone (Godek et al., 1980a; 1980b; and 1980c). The Ames Salmonella mutagenesis assay was used, utilizing strains TZ 1535, TZ 1537, TA 1538, TA 98 and TZ 100. Experiments were done both with and without rat liver microsomal metabolic activation. All assays were negative for the three toxic compounds.

Cambon et al. (1979) fed aldicarb to pregnant female rats at daily doses up to 1.0 mg/kg. They found no teratogenic effects or significant effects on the standard indices of reproduction. Acetylcholinesterase depression was noted in fetuses when pregnant rats were given aldicarb orally by gavage on day 18 of gestation, indicating placental transfer.

Analytical Techniques

A number of methods exist for the detection and analysis of aldicarb and its toxic metabolites. They include: 1) colorimetric methods, 2) gas chromatographic (GC) methods; 3) high performance liquid chromatographic (HPLC) methods and 4) gas chromatography/mass spectrometric (GC/MS) methods.

Colorimetric methods were early techniques which did not distinguish between aldicarb, aldicarb sulfoxide, aldicarb sulfone and other metabolites (Johnson and

Stansbury, 1966 and Kearby et al., 1970). Extracted residues (TTR plus corresponding oximes) were hydrolyzed by base to yield oximes. The oximes were then subjected to acid hydrolysis to release hydroxyl amine which was oxidized with iodine to nitrous acid. Nitrous acid was determined colorimetrically by a nitrite-diazo dye procedure. The color was stable for one hour and sensitivity was approximately 0.03 ppm.

Gas chromatographic methods utilize element specific detectors such as flame photometric detectors operated in the sulfur mode or nitrogen phosphorous (N/P) detectors. Analytical procedures for aldicarb in plants, soil or water generally involve extraction of the matrix with a chlorinated solvent such as chloroform or methylene chloride followed by a column chromatographic clean up, if necessary, and concentration of the extract. Then the sample is injected on an appropriate GC column (Carbowax 20M, DEGS, etc.) and the individual toxic metabolites (aldicarb, aldicarb sulfoxide and aldicarb sulfone) are detected and quantified (actually, thermal decomposition to the corresponding nitriles occurs in the injection port). If only the TTR is desired then the sample can be treated with peracetic acid to convert aldicarb and aldicarb sulfoxide to the sulfone. Then the TTR is detected as sulfone. The sensitivity of these methods is in the range of 0.005 to 0.01 ppm. N/P detectors are generally more

sensitive than flame photometric detectors. (Carey and Helrich, 1970, Maitlen et al., 1969 and Maitlen et al., 1968. Bromilow, R.H., 1973, Andrawes et al., 1973, Halfhill and Maitlen, 1973, Lindquist et al., 1972, Kreuger and Mason, 1974, Woodham et al., 1973a, and Woodham et al., 1973b).

The sample preparation (extraction, concentration, etc.) for HPLC analysis procedures can be similar to those for GC, however, conversion with peracetic acid of aldicarb and aldicarb sulfoxide to the sulfone is generally not done for HPLC. Several approaches have been used for analysis of aldicarb and related products by HPLC. One employs a reverse phase column with acetonitrile : water as the mobile phase and detection by a variable wavelength ultraviolet detector. The detection limit in potatoes, using a Sep-Pak cleanup, was 0.05 ppm (20 ng) for aldicarb and 0.1 ppm (37.5 ng) for sulfoxide and sulfone (Cochrane and Lanovette, 1981). The detection limit in water samples (1.0 L samples) using a Sep-Pak cleanup, was 1.0 ppb for all three compounds (Cochrane et al., 1982). Direct analysis of water samples without prior cleanup yielded the following detection limits (1.0 L size samples) for aldicarb and related compounds: aldicarb, 6 ppb (1.2 ng); aldicarb sulfoxide, 11 ppb (2.2 ng); aldicarb sulfone, 10 ppb (2.0 ng); aldicarb oxime, 5 ppb (1.0 ng); aldicarb sulfoxide oxime, 6 ppb (1.2 ng); aldicarb sulfone oxime,

9 ppb (1.8 ng); aldicarb nitrile, 31 ppb (6.1 ng); aldicarb sulfoxide nitrile, 20 ppb (4.0 ng); aldicarb sulfone nitrile, 6600 ppb (1300 ng) (Miles and Delfino, 1984).

Another popular HPLC method employs post-column derivatization and may be applied generally to the analysis of N-methylcarbamate and carbamoyl oxime pesticides. This method involved post-column hydrolysis of the pesticide to yield methyl amine which was then fluorogenically labeled with o-phthalaldehyde (OPA). The limit of detection was 0.1 ng. The chromatographic separations were performed on reverse phase columns and the OPA-methyl amine complex was detected using a fluorometer (Moye et al., 1977). This method was adapted to a multiresidue approach for determination of N-methylcarbamate pesticides in crops (Krause, 1980) and subjected to a collaborative study in 14 crops with an estimated limit of quantitation of 0.01 ppm (Krause, 1985). This method was also developed to measure N-methyl carbamoyl oximes and N-methyl carbamates in drinking water by direct on column aqueous injection with detection limits dependent on the compound, ranging from 1.0 to 4.1 ppb (Foerst, 1984).

Gas chromatography/mass spectrometry (GC/MS) methods of analysis must contend with the thermal lability of carbamate molecules in general. For analysis of aldicarb, Trehy et al. (1984), used a short capillary column coupled to the mass spectrometer to overcome this problem. The

limits of detection for aldicarb, aldicarb oxime and aldicarb nitrile were 0.3 ng, 1.2 ng and 0.15 ng, respectively. Direct probe chemical ionization (CI)/MS obtained molecular ion + H peaks for aldicarb, aldicarb sulfoxide and aldicarb sulfone with minimal but different fragmentation patterns (Muszkat et al., 1982 and Muszkat and Aharonson, 1982). When GC/MS/CI was used molecular ions were not detected for any of the three compounds, however, they had distinguishable fragmentation patterns (Muszkat and Aharonson, 1983). A liquid chromatography (LC)/MS method was developed for analysis of aldicarb and related products in water. Single ion monitoring (SIM) was used for quantitation; the limits of detection for aldicarb, the sulfoxide and the sulfone were 0.3 ppb, 0.6 ppb and 0.3 ppb, respectively (Wright et al., 1982).

Metabolism of Aldicarb in Plants, Microsomes, Fungi and Rats

Andrawes et al. (1971a) studied the metabolism of aldicarb in greenhouse and field-grown Irish potato plants (Cobbler variety). Three labeled preparations of aldicarb ($-S-^{14}CH_3$, tert.^{14}C and $-NH-^{14}CH_3$) were applied by injection into the stem (greenhouse grown) or by in-furrow application (3.36 kg/ha).

Greenhouse grown potatoes were harvested at 0, 1, 7, 14 and 28 days post injection. No residues were detected in tubers until 7 days post injection; increased between 7 and 14 days and then remained level from 14 to 28 days.

The final level in the tubers was 2% of that in the foliage. Approximately 40-50% of the applied ^{14}C was lost over 28 days. Aqueous soluble metabolites increased rapidly from 0 to 24 hr. and gradually thereafter. The half-life of aldicarb was less than 24 hr. in the foliage. Seventy-six percent of the ^{14}C in the tubers at 28 days was aqueous soluble and only 6.1% of the ^{14}C was aldicarb sulfoxide.

In the foliage, at 24 hr., 70-80% of $CHCl_3$ soluble ^{14}C was aldicarb sulfoxide (20-30% of applied ^{14}C). The half-life of aldicarb sulfoxide was approximately 28 days. The aldicarb sulfone concentration was relatively minor and decreased from 1 (4.4%) to 28 (0.9%) days.

The distribution and percentages of applied radiocarbon in field grown potatoes, harvested 30, 60 and 90 days post application are presented in Table 2-1. The seed piece (portion of a tuber containing an eye from which potato plant will grow) contained the most ^{14}C followed by the foliage, tubers and roots at 30 and 60 days.

Parent aldicarb was not detected in the foliage at any sampling period; aldicarb sulfoxide represented 45% of the ^{14}C at 30 days and decreased to 6.6% (0.29 ppm) at 90 days. There was an increase in aldicarb sulfone from 30 days (25.8%, 1.20 ppm) to 90 days (55.9%, 2.45 ppm). The sulfoxide oxime and sulfone oxime were detected as minor metabolites at 90 days (less than 4%). The tubers

Table 2-1. Percentage of Applied ^{14}C and ppm in Potato Plants.

	ppm			% of Applied ^{14}C		
Plant Part	30	60	90	30	60	90
Foliage	4.66	6.66	4.38	0.56	3.29	2.04
Tubers	-	1.39	0.79	-	0.48	0.95
Roots	3.25	2.51	2.31	0.12	0.16	0.04
Seed piece	36.90	10.60	-	3.17	0.13	-

from Andrawes et al. (1971a).

contained similar metabolites, but the relative concentrations varied. At the 60 day interval, equal quantities of water soluble metabolites, aldicarb sulfoxide and aldicarb sulfone were detected. The major water soluble metabolites were glycoside conjugates of the oxime, the sulfoxide and sulfone alcohol.

Aldicarb metabolism has also been reported in sugar beets and cotton. Rouchaud et al. (1980a, 1980b and 1981) reported on the distribution and metabolism in field grown sugar beet plants treated at 3 kg/ha (normal rate for sugar beets is 1 kg/ha). Plants were separated into blades and petioles of internal and external leaves, crowns and roots. The ppm of ^{14}C as aldicarb equivalents was: external leaf blades (0.93 ppm), internal leaf blades (0.68 ppm), external leaf petiole (0.26 ppm), internal leaf petiole (0.05 ppm), crowns (0.15 ppm) and roots (0.13 ppm). TTR's were present only in the leaf blades at concentrations of 0.13 to 0.23 ppm. No TTR were detected in the crowns or roots. Unextractable materials were highest in the petioles, crowns and roots (approximately 40%). Major metabolites in the leaves were the sulfone alcohol (49.2%) and aldicarb sulfone (14.2%). Thirty-seven percent of the sulfone alcohol was present as water soluble conjugated species. Minor amounts of aldicarb sulfoxide (0.2%), aldicarb sulfoxide oxime (0.5%), aldicarb sulfone oxime (1.2%), aldicarb sulfoxide nitrile

(0.8%), aldicarb sulfone nitrile (2.6%) and aldicarb sulfoxide alcohol (0.3%) were also detected. Seven percent of the ^{14}C was unextractable. The petioles showed a similar variety of metabolites; however, 42% of the ^{14}C was unextractable.

The metabolism of aldicarb in cotton was studied by Coppedge et al. (1967), Bartley et al. (1970) and Andrawes et al. (1973). Metabolites similar to those found in potatoes and sugar beets were detected in all experiments. However, Bartley et al. (1970) further investigated the aqueous soluble fractions of the plant extracts which represented the major portion at the final harvest period. The primary water soluble metabolite was a conjugate of 2-methyl-2-(methyl sulfinyl)-propanol (70-80%). Smaller quantities of conjugates formed with 2-methyl-2-(methyl sulfonyl) propanol, 2-methyl-2-(methyl sulfonyl) propionaldehyde oxime were also found. Non-conjugated species found were 2-methyl-2-(methyl sulfinyl) propionamide, 2-methyl-2-(methyl sulfinyl) propionic acid and 2-methyl-2-(methyl sulfonyl) propionic acid.

Metabolism studies have also been carried out in plant microsomes and soil fungi. Again, metabolites similar to those found in the in vivo studies were detected. In vitro formation of aldicarb sulfoxide with soybean root homogenates from aldicarb was reported by Krueger (1977). Oxidation of aldicarb to the sulfoxide occurred to the

greatest extent in the 105,000 g supernatant and in the 25,000 g supernatants. The 25,000 g supernatant fraction of the soybean root was found to be the most metabolically active portion of the plant followed by the stem, cotyledon and leaf.

Jones (1976) used pure cultures of five common soil fungi to study the degradation of aldicarb. There was considerable variability among the fungi in the amounts of organic and water soluble materials formed. The major organic soluble materials were aldicarb sulfoxide, and the sulfoxide oxime and nitrile sulfoxides, with small amounts of the corresponding sulfones. The primary water soluble metabolites were the sulfone alcohol and amide with smaller amounts of the sulfoxide alcohol and amide.

Andrawes et al. (1967) evaluated the metabolism and elimination of aldicarb in rats. Aldicarb was administered orally and 80% was eliminated through the urine within 24 hours. Four percent was detected in the feces. Chloroform extraction of the urine yielded aldicarb oxime, the sulfoxide oxime, the sulfone oxime, the sulfoxide nitrile, the sulfone nitrile, the sulfoxide aldehyde oxime and the sulfone aldehyde oxime. Nearly half of the urine metabolites remained water soluble and unidentified. They were most likely glucuronide and other conjugates of the alcohol metabolites and other conjugates.

Behavior of Aldicarb in Soil

After aldicarb was detected in ground water much data have been published describing the behavior of aldicarb in soil. The movement of the TTR in soil columns, the metabolic and hydrolysis rates as well as other losses, and the carry over properties to crops have all been described. Such data allow predictions to be made concerning the environmental fate of the chemical.

Awad et al. (1984) studied the movement of aldicarb in different soil types under laboratory conditions. Percolation of large volumes of water on the movement of the compounds was one of the factors evaluated. The results differed considerably with respect to soil type. Movement was most rapid in sandy soil and slowest in clay soils; the highest levels of aldicarb were found in water leached from sandy, sandy-loam and peat soils, and the lowest levels were found in the leachates from clay and loam soils.

The metabolic rates of aldicarb, aldicarb sulfoxide and aldicarb sulfone in "plough layers" and two deeper layers were determined by Smelt et al. (1978a; 1978b; 1978c). The loss of aldicarb was described by first order kinetics. A large percentage (91-100%) of aldicarb was converted to the sulfoxide in 10 to 30 days; the time depended on the soil type. Clay loams showed more rapid conversion than did sandy soils. The half-life for

aldicarb sulfoxide ranged from 20 days in clay loam to 46 days in peaty sand and could also be described by first order kinetics. Loss of aldicarb sulfoxide in the deeper layers was slower. In a sandy layer 90-110 cm deep, for example, only 15% was lost after 111 days. The conversion of aldicarb sulfone could also be described by first order kinetics for the first two to three half-lives. These half-lives ranged from 18 days in a clay loam to 154 days in a peaty sand. Slower conversion again was observed in the deeper layers. In fact, no loss was observed in a sand layer at 90-110 cm during 294 days of incubation.

Characteristics of the volatilization of aldicarb from soil were reported by Supak et al. (1977). Moisture and temperature effects on volatilization and degradation were studied. Volatilization was generally suppressed by the presence of water and elevated temperatures. Degradation was more rapid as the temperature was increased and moisture decreased.

To evaluate the potential for aldicarb to remain in the soil and contaminate subsequent crops, Andrawes et al. (1971b) applied Temik at a rate of 3.4 kg/ha in furrow. This resulted in ^{14}C residues of approximately 15 ppm in the soil. By 90 days after application the levels had decreased to about 0.06 ppm (as total ^{14}C aldicarb equivalents). Heavy rainfall enhanced dissipation rates. Aldicarb sulfoxide and aldicarb sulfone water soluble

metabolites were the major products detected. Tomato plants, grown for 7 days in the soil, 90 days after application, contained 0.01 to 0.06 ppm total ^{14}C residues. They also reported that volunteer crab grass growing in the treated field contained 1.15 ppm of ^{14}C residues.

General Conclusions

The major mechanism for contamination of the environment by aldicarb residues is through agricultural practices. Experiments with soils indicate that aldicarb is extensively degraded and dissipated under certain conditions, particularly in the upper soil layers. There is also evidence, however, that under certain conditions the half life of aldicarb TTR is prolonged which coupled with rapid water percolation through soil could lead to ground water contamination. Metabolic data from various biological systems show rapid degradation of the toxic components. Generally TTR are low to non-detectable in the edible portions of plants. Elimination in mammals is rapid and acetylcholinesterase inhibition is reversible. Acute toxicity appears to be the major concern to man from exposure.

CHAPTER III

METABOLISM OF ALDICARB, ALDICARB SULFOXIDE AND ALDICARB SULFONE IN ATLANTIC VARIETY POTATO PLANTS

Materials and Methods

Chemicals

Radiochemicals. S-methyl [^{14}C] aldicarb (specific activity 6.06 mCi/mM) and S-methyl [^{14}C] aldicarb sulfone (specific activity 7.1 mCi/mM) were gifts of Union Carbide Corporation. S-methyl [^{14}C] aldicarb sulfoxide was prepared from S-methyl [^{14}C] aldicarb by peracetic acid oxidation (Payne et al., 1966). The reaction was performed by stepwise addition of peracetic acid to an equimolar solution of [^{14}C] aldicarb in ethyl acetate (32-37°C) over a 60 minute period. The extent of completion of the reaction was followed by thin layer chromatography (TLC). When the reaction was essentially complete, water was added and the mixture was partitioned four times with chloroform. The chloroform fractions were combined and concentrated. The [^{14}C] aldicarb, [^{14}C] aldicarb sulfoxide and [^{14}C] aldicarb sulfone were purified by TLC.

Analytical standards and enzymes. Analytical reference standards furnished by Union Carbide were aldicarb (T), aldicarb sulfoxide (T_1), aldicarb sulfone (T_2), aldicarb sulfoxide oxime (T_2O), aldicarb sulfoxide nitrile (T_1N) and aldicarb sulfone nitrile (T_2N). ß-Glucosidase from almonds and hemicellulase from Aspergillus niger were used as received from Sigma Chemical Co.

Most solvents used were Fisherbrand pesticide grade. In some cases technical grade chloroform was redistilled twice prior to use. All water was deionized and carbon filtered.

Chromatographic Procedures

Thin layer chromatography. TLC was done on silica gel 60 F-254 chromatoplates (0.25 mm X 20 cm, E. Merck) utilizing the following solvent systems: (A) chloroform: n-hexane:ethyl acetate:ethanol (5:1:1:1); (B) methylene chloride:acetonitrile (3:1); (C) chloroform:methanol (6:1); (D) diethyl ether:n-hexane:ethanol (4:1:1); (E) benzene: methanol:glacial-acetic acid (4:1:1). The chemical names, common names, abbreviations and TLC R_f values for aldicarb and related compounds in the indicated solvent systems are listed in Table 3-1.

Ion exchange chromatography. The aqueous phases were concentrated on a rotary evaporator to approximately 5 mL prior to analysis. They were subjected to ion exchange chromatography (in a manner similar to that used by Bartley

Table 3-1. Thin-Layer Chromatographic Behavior of Aldicarb and Related Standards.

Compound(Common Name)	Abbreviation	Rf Value in Indicated Solvent System[a] A	B	C	D	E
2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb)	T	0.92	0.85	0.95	0.98	0.85
2-Methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb sulfoxide)	T1	0.45	0.10	0.68	0.24	0.89
2-Methyl-2-(methylsulfonyl)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb sulfone)	T2	0.61	0.61	0.79	0.59	0.55
2-Methyl-2-(methylsulfinyl)propionaldehyde oxime(Sulfoxide oxime)	T1 O	0.52	0.14	0.59	0.73	0.67
2-Methyl-2-(methylsulfonyl)propionaldehyde oxime(Sulfone oxime)	T2 O	0.73	0.74	0.75	0.95	0.73
2-Methyl-2-(methylsulfinyl)propionitrile (Sulfoxide nitrile)	T1 N	0.68	0.74	0.85	0.64	0.62
2-Methyl-2-(methylsulfonyl)propionitrile (Sulfone nitrile)	T2 N	0.85	0.67	0.92	0.88	0.70
2-Methyl-2-(methylsulfinyl)propionamide (Sulfoxide amide)	T1 AM	0.36	0.09	0.60	-	-
2-Methyl-2-(methylsulfonyl)propionamide (Sulfone amide)	T2 AM	0.53	0.35	0.71	-	-
2-Methyl-2-(methylsulfinyl)propanol (Sulfoxide alcohol)	T1 AL	0.23	0.07	0.49	-	-
2-Methyl-2-(methylsulfonyl)propanol (Sulfone alcohol)	T2 AL	0.63	0.61	0.65	-	-
2-Methyl-2-(methylsulfinyl)propionaldehyde O-carbamoyloxime(Demethyl sulfoxide)	DT1	-	0.08[b]	0.47[b]	0.12[c]	-
2-Methyl-2-(methylsulfonyl)propionaldehyde O-carbamoyloxime(Demethyl sulfone)	DT2	-	0.41	0.58[b]	0.45[c]	-
2-Methyl-2-(methylthio)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT	-	0.55[b]	0.78[b]	-	-
2-Methyl-2-(methylsulfinyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT1	-	0.00[b]	0.42[b]	-	-
2-Methyl-2-(methylsulfonyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT2	-	0.29[b]	0.62[b]	-	-
2-Methyl-2-(methylsulfinyl)propionic acid (Sulfoxide acid)	T1 AC	-	0.00	Streaked[b]	-	0.70[b]
2-Methyl-2-(methylsulfonyl)propionic acid (Sulfone acid)	T2 AC	-	0.00	Streaked[b]	-	0.72[b]
N-hydroxy-2-methyl-2-(methylsulfinyl)-propionamide	T1 HA	-	0.00[b]	Streaked[b]	-	0.29[b]
N-hydroxy-2-methyl-2-(methylsulfonyl)-propionamide	T2 HA	-	0.00[b]	Streaked[b]	-	0.51[b]

[a] A, chloroform:hexane:ethyl acetate:ethanol (5:1:1:1); B, methylene chloride:acetonitrile (3:1); C, chloroform:methanol (6:1); D, diethyl ether:n-hexane:ethanol (4:1:1); E, benzene:methanol:acetic acid (4:1:1).

[b] From Bartley et al., 1970.

[c] From Coppedge et al., 1967.

et al., 1970) on Dowex 1 X 8 - 50 anion exchange resin in 1.2 cm ID X 23 cm columns. Approximately 1.0 mL of the aqueous sample was applied to the column and eluted using ammonium carbonate buffer (adjusted to pH 6.8 with CO_2). Eluants were combined and concentrated to dryness for analysis by TLC or for enzymatic hydrolysis.

Mass Spectrometry. Mass spectra of reference standards were recorded on a Finnigan 4021 Gas Chromatograph/Mass Spectrometer (GC/MS) operated in the electron impact (EI) mode. Pure samples of T, T1, T10, T2, T20 and T2N were analyzed by direct inlet probe. Probe temperature was increased from 40°C to 350°C until volatilization of the compounds occurred. Source temperature was 250°C, the emission current was 0.35 mA and electron energy was 70 eV. A sample of T1N was analyzed by GC/MS on 15 m x 0.32 mm DB-5 capillary column (J & W) temperature programmed from 50°C to 200°C at 10°C/min. The injection port temperature was 200°C and other MS conditions were as described above.

The spectra from these analyses are shown in the Appendix (Figures A-1, A-2, A-3, A-4, A-5, A-6, A-7 and A-8). The T, T1, T10 and T20 spectra matched reference spectra in the on line National Bureau of Standards library in the Finnigan 4021 GC/MS/DS and were similar to published EI and CI spectra (Benson and Damico, 1968, Muszkat and Aharanson, 1982 and Wright et al., 1982). The library chose T20 to match with the spectrum for T2, however,

comparison of the T2 spectrum with the library T2 spectrum showed similar major ions. Reference spectra for T1N and T2N were not in the library, however, they show characteristic nitrile fragmentation.

Enzymatic Hydrolyses

The glycosides isolated from the ion exchange column were taken up in 5 mL of 0.1 M sodium acetate buffer (pH 5.0) containing 125 mg of ß-glucosidase and 125 mg of hemicellulase. One drop of toluene was added and the mixture incubated overnight on an orbital shaker at 37°C. The solution was partitioned four times with chloroform and both chloroform and aqueous fractions analyzed by TLC.

Plant Growth and Treatment

The Atlantic variety potato (Solanum tuberosum) was used. Whole seed potatoes were planted in 32 cm pots (one seed per pot) containing washed white sand. The seeds were germinated in a greenhouse and were transferred to environmental growth chambers (Scherer-Gillet Model CEL 512-37) two weeks post germination. Light and dark periods were 12 and 12 hours, respectively; light and dark temperatures were 29° and 19°C, respectively; light and dark humidities were 80% and 60%, respectively. The plants were watered with a Hoaglands nutrient solution as necessary.

The potatoes were treated with labeled pesticides two to three weeks post germination. The formulation (Temik 15G) and ^{14}C materials were applied through a small hole

bored in the sand down to the seed potato. The formulation granules were placed in contact with the seed potato and 10 mL of H_2O was pipetted on the granules. Then 1.0 mL of the ^{14}C material in acetone was pipetted on the granules followed by 10 mL of water. The hole was refilled with sand and the plants were watered. Each pot received an application rate equivalent to 3.36 kg/ha of formulation (0.18 g containing 27.04 mg active ingredient) and either 9.6 uCi of S-methyl [^{14}C] aldicarb (specific activity 7.81 x 10^5 dpm/mg), 10.0 uCi of S-methyl [^{14}C] aldicarb sulfoxide (specific activity 8.14 x 10^5 dpm/mg), or 11.0 uCi of S-methyl [^{14}C] aldicarb sulfone (specific activity 8.95 x 10^5 dpm/mg). Untreated control plants were also maintained in the growth chambers.

Plant and Sand Analysis

The potato plants were harvested approximately 2, 6 and 10 weeks post application (4, 8 and 12 weeks post germination, respectively). At each harvest two plants were analyzed. The total fresh weight and the weights of the various plant parts at each harvest for plants treated with aldicarb, the sulfoxide or the sulfone are shown in Table 3-2. Plants were subdivided for analysis into foliage, stem, seed potato, tubers and stolons and roots. Two week post application plants had no tubers or stolons. Control plants were grown in the same growth chamber as the treated plants.

Table 3-2. Fresh Weight in Grams of Plant Parts at Three Harvest Intervals Post Application for Plants Treated with ^{14}C-Aldicarb, ^{14}C-Aldicarb Sulfoxide and ^{14}C-Aldicarb Sulfone.

	Aldicarb			Sulfoxide			Sulfone		
Plant Part	2 [a]	6	10	2	6	10	2	6	10
Foliage	37.44[b] (16.77)[c]	80.29 (38.12)	313.00 (89.00)	57.16 (1.06)	73.25 (3.23)	103.50 (5.70)	24.51 (2.03)	54.39 (0.50)	94.31 (2.18)
Stem	27.64 (9.33)	104.13 (55.79)	348.11 (131.88)	60.83 (1.32)	72.75 (7.48)	107.00 (7.10)	26.94 (0.94)	29.86 (0.32)	47.04 (1.18)
Seed	101.80 (21.61)	134.72 (3.94)	13.63 (2.05)	178.08 (41.93)	53.67 (2.64)	54.24 (0.94)	118.54 (4.51)	116.39 (2.24)	125.93 (6.80)
Tuber	-	20.63 (0.38)	283.01 (166.60)	-	51.88 (2.65)	269.19 (9.00)	-	98.93 (7.77)	237.10 (12.51)
Root	16.21 (8.21)	36.93 (8.90)	141.93 (25.02)	17.04 (8.82)	38.06 (2.21)	63.97 (3.10)	14.75 (2.91)	18.17 (6.33)	20.08 (0.38)
Total	183.09 (3.72)	376.70 (107.14)	1099.68 (410.46)	313.11 (51.01)	289.61 (8.51)	597.94 (14.44)	184.74 (6.33)	317.74 (2.86)	524.47 (9.45)

[a] Weeks post application.

[b] Mean of two plants.

[c] Standard deviation.

The various plant parts were cut into 1.0 to 2.0 cm pieces and homogenized in a Waring blender for 10 min. with ethanol:water (1:1) (2 mL solvent per gram of tissue). The blended extracts were centrifuged and the supernatant suction filtered through scintered glass funnels. Plant tissues remaining were twice resuspended in ethanol:water (1:1), centrifuged and suction filtered. These crude extracts were assayed for ^{14}C, then reduced in volume using a rotary evaporator at 40°C to a volume of approximately 40 mL and assayed for ^{14}C. After concentration the extracts were transferred to separatory funnels and partitioned according to the procedure of Andrawes et al. (1971). An equivalent volume of acetonitrile was added to the aqueous extracts and this mixture was partitioned four times with chloroform (the volume of chloroform aliquots were one-half the volume of the concentrated extract plus acetonitrile). The aqueous phase and combined chloroform aliquots were assayed for ^{14}C. The organic phases were concentrated using a rotary evaporator to approximately 10 mL for a ^{14}C determination and TLC analysis of the ^{14}C components.

The sand in the treated pots was separated into 3 layers; the top 7.5 cm, the middle 7.5 cm and the bottom 7.5 cm. Each layer was mixed to homogeneity and approximately one third of the volume of each layer was placed in a 1000 mL buret and 4 x 250 mL of acetone were percolated through the column. The extracts were

concentrated on rotary evaporators. These concentrated extracts were partitioned and further analyzed as described above for the plants.

Radiochemical Analysis

All liquid scintillation counting (LSC) was done using a Searle Analytic 92 liquid scintillation counter utilizing Aquasol II plus 10% water as the scintillation cocktail. The cpm were converted to dpm using quench curves. Tissues which remained after extraction were dried at 105°C for 24 hr. Radiocarbon determinations were made by combustion using a Packard Tri-Carb Sample Oxidizer B306.

Identification of Conversion Products

Organic phase concentrates were spotted on TLC plates and developed to 10 cm. Areas of ^{14}C were detected by placing the plates in contact with Kodak SB-5 X-ray film for autoradiography. Each ^{14}C spot was scraped, eluted with scintillation cocktail and the radioactivity counted to determine the percentage distribution of ^{14}C. Radiolabeled standards of aldicarb, aldicarb sulfoxide and aldicarb sulfone were spotted on all sample TLC plates. The identity of the non-labeled reference standards (T, T1, T2, T1O, T2O, T1N and T2N) was confirmed by EI/MS as described above.

The R_f values of the above reference compounds were directly compared to sample R_f values in the 5 TLC solvent systems for identification. Identification of the

alcohols, amides and acids was by comparison of sample R_f values to literature R_f values (Bartley, et al., 1970 and Coppedge et al., 1967).

Statistical Methods

All data were expressed as averages of duplicate samples and standard deviations were calculated. Analysis of variance (one-way) using a completely randomized design was performed on selected sets of data to determine if the means were equal. In each case treatment sums of squares (SS), total SS and error SS were calculated and used to determine the F statistic which was compared to a Table F value (Steel and Torrie, 1960).

Results

Total Distribution of Radiocarbon

The total radiocarbon content of plant foliage, stem, seed, tuber and root and three sand layers was determined. These components represent potential compartments in which the applied radiocarbon can be distributed. These data can be examined in various ways to illustrate the distribution of radiocarbon among these compartments. Comparisons which will be made include: distribution of radiocarbon between the whole plant and sand, distribution within the plant, and distribution in three sand layers.

Radiocarbon distribution between plant and sand. The distribution of radiocarbon between the plant and sand was determined by combining the total ^{14}C in the various plant

components and the total ^{14}C in the three sand layers. This distribution can be expressed in two ways; the radiocarbon distribution can be expressed in terms of the percentage of the applied radiocarbon, and the radiocarbon distribution can be expressed as percentages of the recovered ^{14}C.

The distribution of radiocarbon between the whole plant and sand as a percentage of the applied ^{14}C at three post application harvest periods for plants treated with aldicarb, aldicarb sulfoxide and aldicarb sulfone is presented in Table 3-3. The table also shows the total radiocarbon recovery. Plants treated with aldicarb and the sulfoxide contained 40 to 55% of the applied ^{14}C at all harvest intervals. Sulfone treated plants contained a smaller percentage of the applied ^{14}C, between 24 and 38 percent. Analysis of variance of the percentage ^{14}C in the plant aldicarb, the sulfoxide and the sulfone yielded calculated F statistics of $F = 2.05$, $F = 0.11$ and $F = 1.65$, respectively. The F statistic from the F-table at a 0.05 confidence level was $F_{0.05,2,3} = 9.55$. Consequently, one may accept the hypothesis that the means from the three aldicarb harvests are the same, the means from the three sulfoxide harvests are the same and the means of the percentage radiocarbon in the plant from the three sulfone harvests are the same.

Table 3-3. Total Percentage Radiocarbon Recovered, of the Applied, in the Whole Plant and Sand at Three Harvest Intervals Post Application from Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone Experiments.

	Aldicarb			Sulfoxide			Sulfone		
	1 [a]	2	3	1	2	3	1	2	3
Plant	42.3[b] (6.5)[c]	40.5 (0.6)	49.7 (5.2)	50.9 (1.1)	50.1 (4.0)	53.8 (11.3)	28.6 (13.0)	38.0 (3.4)	23.7 (1.5)
Sand	31.0 (2.7)	11.7 (4.7)	9.9 (5.9)	34.2 (6.3)	20.0 (5.8)	6.3 (2.9)	47.3 (12.9)	12.5 (1.7)	8.8 (3.5)
Total Recovered	73.3 (9.1)	52.2 (4.1)	59.6 (0.8)	85.1 (7.4)	70.1 (9.8)	60.1 (8.4)	75.9 (0.2)	50.5 (5.1)	32.5 (2.0)

[a] Weeks post application.

[b] Mean of duplicate samples.

[c] Standard deviation.

The percentage of the applied ^{14}C in the sand decreased as time post application increased for all three compounds, 30% to 48% at 2 weeks to less than 10% at 10 weeks. Analysis of variance of the means of the percentage of applied ^{14}C in the sand for the three harvests yielded calculated F statistics of 12.82, 14.11 and 15.01 for the aldicarb, aldicarb sulfoxide and aldicarb sulfone experiments, respectively. Since the table F statistic was $F_{0.05,2,3} = 9.55$, the percentages of applied ^{14}C in the sand are not the same throughout the harvest periods for all three compounds. Therefore, the apparent decrease in applied ^{14}C in the sand as time post application increased is statistically significant. There was also a decrease in the percentage of total ^{14}C recovered as time post application increased for all three compounds.

Radiocarbon distribution within the plants. The distribution of radiocarbon in the plant (foliage, stem, seed, tuber and root) as a percentage of the recovered radiocarbon at three post application harvest periods for plants treated with aldicarb, the sulfoxide and the sulfone is presented in Table 3-4. For all compounds at all harvest intervals the foliage contained most of the ^{14}C in the plant. The percentage of ^{14}C in the foliage for all compounds ranged from 70 to 80% at 2 weeks and increased to 90 to 94% at 10 weeks. The seed potato contained the next largest percentage of recovered ^{14}C in the plant, however,

Table 3-4. Percentage Distribution of Recovered Radiocarbon in Various Plant Parts at the Three Post Application Harvest Intervals for Plants Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone.

	Aldicarb			Sulfoxide			Sulfone		
Plant Part	2 [a]	6	10	2	6	10	2	6	10
Foliage	71.1[b] (25.9)[c]	79.1 (5.6)	93.3 (1.2)	82.7 (5.4)	86.5 (11.5)	87.8 (5.5)	93.8 (4.5)	93.0 (0.1)	91.5 (0.2)
Stem	3.6 (0.4)	3.7 (1.3)	2.3 (1.2)	4.4 (1.2)	3.8 (1.0)	5.9 (2.4)	1.2 (0.7)	1.7 (0.7)	1.7 (0.1)
Seed	24.7 (25.3)	15.9 (7.0)	1.0 (0.3)	9.4 (2.3)	5.6 (7.9)	2.2 (1.1)	4.4 (3.4)	3.2 (0.8)	3.2 (0.01)
Tuber	-	0.6 (0.3)	1.3 (0.5)	-	1.5 (1.7)	2.9 (0.5)	-	1.6 (0.1)	2.9 (0.3)
Root	0.6 (0.3)	0.7 (0.4)	2.1 (0.2)	3.5 (1.9)	2.6 (1.9)	1.2 (0.9)	0.6 (0.4)	0.5 (0.1)	0.7 (0.01)

[a] Weeks post application.

[b] Mean of duplicate samples.

[c] Standard deviation.

there was a decrease in the percentage as time post application increased for all three compounds. There was 4.0 to 25.0% at 2 weeks and 1.0 to 3.0% at 10 weeks. The stem ^{14}C remained relatively constant throughout the harvest periods ranging from 1.0 to 5.0%. Root ^{14}C was low and ranged between 0.5 to 3.5% for all compounds at all harvest periods. Tubers, not present at 2 weeks, contained a relatively small percentage of the plant ^{14}C at 6 weeks; tuber ^{14}C content approximately doubled between 6 and 10 weeks for all compounds. The percentages ranged from 0.6 to 2.9%. In general, there were no major differences in the percentage distribution of ^{14}C among the plant parts for plants treated with aldicarb, the sulfoxide and the sulfone. At 10 weeks, which corresponds to the normal harvest period for mature potatoes, the percentage distribution in the plant in descending order was foliage >> stem $\geq$ seed $\geq$ tuber $\geq$ root.

The percentages in Table 3-4 represent recovered ^{14}C in the plant. The data from Tables 3-3 and 3-4 can be used to calculate the percentage distribution as a function of the applied radiocarbon. For example, to determine the percentage of the applied ^{14}C recovered in the foliage of aldicarb treated plants at the first harvest, multiply the percentage (for the whole plant) in Table 3-3, 42.4, times the percentage (for the plant part) in Table 3-4, 0.710 to get 30.1% of applied in the foliage.

The concentration of ^{14}C residues in terms of ppm in the various plant parts expressed as ^{14}C aldicarb equivalents is presented in Table 3-5 for potato plants treated with aldicarb, the sulfoxide and the sulfone for the three post application harvest periods. Also indicated in Table 3-5 is the total ppm in the plant as ^{14}C aldicarb equivalents. This value was lower than the sum of all the individual plant part ppm due to the contribution of weight from certain plant parts (particularly the seed and stem) which contained relatively small amounts of total ^{14}C residue. The ppm levels were generally high at 2 weeks and had decreased by 10 weeks in all plant parts for all compounds.

Radiocarbon distribution in the sand. The percentage distribution of recovered radiocarbon in the three sand layers at the three post application harvest periods for all three experiments is shown in Table 3-6. By 10 weeks ^{14}C had distributed throughout the pot for all three compounds. Generally, the top layer contained the highest percentage of ^{14}C, followed by the middle and bottom layers. As illustrated in Table 3-3, there was a decrease in total sand ^{14}C as time post application increased for all three compounds.

The concentration of total ^{14}C residues, in terms of ppm in the three sand layers, expressed as ^{14}C aldicarb equivalents, is presented in Table 3-7 for pots treated

Table 3-5. Concentration of ^{14}C (ppm) in Plant Parts as Aldicarb Equivalents at Three Post Application Harvest Intervals for Potato Plants Treated with ^{14}C-Aldicarb, ^{14}C-Aldicarb Sulfoxide and ^{14}C-Aldicarb Sulfone.

	Aldicarb			Sulfoxide			Sulfone		
Plant Part	2 [a]	6	10	2	6	10	2	6	10
Foliage	235.0[b] (25.7)[c]	135.6 (57.9)	44.6 (16.0)	198.8 (2.5)	168.1 (14.8)	125.9 (13.8)	282.2 (78.7)	175.6 (12.9)	62.1 (4.2)
Stem	16.6 (5.0)	4.7 (1.5)	0.9 (0.1)	9.8 (1.9)	6.8 (1.4)	7.1 (0.9)	4.6 (0.3)	5.9 (2.1)	2.3 (0.1)
Seed	23.0 (12.2)	13.0 (4.3)	10.0 (1.3)	7.4 (0.4)	8.6 (0.8)	4.6 (0.2)	4.0 (0.2)	2.9 (0.4)	1.6 (0.02)
Tuber	-	3.1 (1.1)	0.8 (0.3)	-	3.5 (0.4)	1.2 (0.1)	-	0.5 (0.1)	0.2 (0.02)
Root	5.1 (1.6)	2.0 (0.4)	2.1 (0.7)	30.7 (4.6)	4.3 (0.5)	1.8 (0.2)	4.1 (0.7)	10.3 (4.4)	9.2 (1.2)
Total	62.7 (8.0)	31.6 (8.7)	14.6 (6.3)	45.0 (6.7)	46.9 (3.3)	24.1 (0.5)	42.4 (4.9)	32.3 (1.8)	12.2 (0.8)

[a] Weeks post application.

[b] Mean of duplicate samples.

[c] Standard deviation.

Table 3-6. Percentage Distribution of Recovered Radiocarbon in Three Sand Layers at the Three Post Application Intervals for Pots Containing Plants Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone.

	Aldicarb			Sulfoxide			Sulfone		
Sand Layer	2 [a]	6	10	2	6	10	2	6	10
Top[b]	93.4[e] (1.7)[f]	58.9 (4.9)	52.8 (17.6)	33.3 (29.2)	67.8 (1.2)	33.4 (1.3)	34.9 (9.9)	55.9 (4.5)	58.7 (12.2)
Middle[c]	5.9 (1.9)	30.9 (4.1)	28.4 (0.3)	30.5 (5.9)	14.0 (0.5)	30.9 (3.1)	57.5 (6.9)	20.3 (2.7)	19.4 (4.7)
Bottom[d]	0.7 (0.1)	10.2 (0.8)	18.8 (17.8)	36.2 (35.1)	18.2 (1.7)	35.7 (4.3)	7.6 (3.0)	23.8 (1.8)	22.1 (7.5)

[a] Weeks post application.

[b] Top 7.5 cm.

[c] Middle 7.5 cm.

[d] Bottom 7.5 cm.

[e] Mean of duplicate samples.

[f] Standard deviation.

Table 3-7. Concentration of ^{14}C (ppm) in Sand Layers as Aldicarb Equivalents at Three Post Application Harvest Intervals for Pots Treated with ^{14}C-Aldicarb, ^{14}C-Sulfoxide and ^{14}C-Sulfone.

	Aldicarb			Sulfoxide			Sulfone		
Sand Layer	2 [a]	6	10	2	6	10	2	6	10
Top[b]	0.967[e] (0.009)[f]	0.230 (0.056)	0.245 (0.144)	0.612 (0.418)	0.570 (0.028)	0.087 (0.003)	0.586 (0.172)	0.252 (0.008)	0.221 (0.083)
Middle[c]	0.062 (0.004)	0.131 (0.031)	0.110 (0.047)	0.542 (0.170)	0.146 (0.006)	0.082 (0.005)	0.985 (0.130)	0.096 (0.010)	0.057 (0.006)
Bottom[d]	0.007 (0.002)	0.045 (0.021)	0.038 (0.013)	0.328 (0.195)	0.272 (0.047)	0.075 (0.011)	0.100 (0.006)	0.100 (0.019)	0.052 (0.003)
Total	0.335 (0.036)	0.136 (0.041)	0.118 (0.050)	0.467 (0.071)	0.353 (0.027)	0.080 (0.002)	0.526 (0.095)	0.148 (0.009)	0.100 (0.027)

[a] Weeks post application.

[b] Top 7.5 cm.

[c] Middle 7.5 cm.

[d] Bottom 7.5 cm.

[e] Mean of duplicate samples.

[f] Standard deviation.

with aldicarb, sulfoxide and sulfone. For all harvest intervals in all three experiments, the ppm levels were highest in the top layer except for the first harvest in the sulfone experiment where the middle layer had the highest levels. There was a decrease in ppm in all layers for all compounds and the total ppm in the pots over the 2 to 10 week interval.

Extractable Radiocarbon

Plant radiocarbon. The percentage distribution of organic soluble metabolites in the various plant parts of aldicarb treated plants at 2, 6 and 10 weeks are presented in Tables 3-8, 3-9 and 3-10, respectively. The tables also show aqueous soluble and unextractable ^{14}C. An explanation of the compound abbreviations used is presented in Table 3-1. Each percentage is the average of two plant part samples carried through the extraction procedure. At 2 weeks post application for aldicarb treated plants, the percentage of radiocarbon which was parent aldicarb had decreased to 2.3%, 6.0% and 26.6% in the foliage, stem and seed, respectively. There was no aldicarb detected in the root extracts. The major component of the extracts from all plant parts (except the seed) was the sulfoxide, which composed 55% to 63% of the recovered ^{14}C. Sulfone percentages detected in the foliage and seed were 5.6% and 3.0%, respectively. Small amounts of the sulfoxide oxime, 1.38%, and sulfoxide nitrile were detected in the foliage.

Table 3-8. Percentage Distribution of Metabolites at Two Weeks Post Application in Potato Plants Treated with Aldicarb.

	Aldicarb Two Weeks				
Compound	Foliage	Stem	Seed	Tuber[a]	Root
T	2.3[b] (1.7)[c]	6.0 (4.0)	26.5 (8.8)	-	-
T1	55.8 (6.6)	62.6 (3.3)	18.1 (6.3)	-	54.7 (8.9)
T2	5.6 (1.5)	-	3.0 (0.01)	-	-
T1 0	1.4 (0.2)	-	0.4 (0.4)	-	-
T1 AM	-[d]	-	-	-	-
T2 AM	-	-	-	-	-
T1 N	3.1 (1.9)	-	-	-	-
T2 N	-	-	-	-	-
T1 Alc	-	-	-	-	-
Unknown 1	-	-	-	-	-
Polar	-	-	-	-	-
Aqueous	27.5 (7.2)	24.5 (0.2)	44.5 (10.1)	-	22.0 (4.4)
Unextractable	4.3 (1.4)	6.9 (0.8)	7.5 (5.5)	-	23.3 (2.4)

[a] No tubers at first harvest.

[b] Mean of duplicate samples.

[c] Standard deviation

[d] None detected

Table 3-9. Percentage Distribution of Metabolites at Six Weeks Post Application in Potato Plants Treated with Aldicarb.

	Aldicarb Six Weeks				
Compound	Foliage	Stem	Seed	Tuber	Root
T	0.4[a] (0.4)[b]	-	1.4 (1.4)	-	-
T1	50.5 (2.0)	38.4 (3.5)	48.3 (1.4)	32.2 (0.9)	12.6 (0.1)
T2	7.6 (0.1)	5.4 (1.2)	1.6 (0.2)	-	-
T1 0	1.5 (0.5)	-	0.4 (0.4)	-	-
T1 AM	0.6 (0.1)	-	0.1 (0.1)	-	-
T2 AM	-[c]	-	-	-	-
T1 N	1.5 (0.04)	-	-	-	-
T2 N	-	-	-	-	-
T1 Alc	0.2 (0.1)	0.3 (0.3)	-	-	-
Unknown 1	-	-	-	-	-
Polar	-	-	-	-	-
Aqueous	26.5 (0.03)	38.5 (5.6)	46.1 (1.0)	55.1 (4.9)	24.9 (9.8)
Unextractable	11.2 (2.0)	17.4 (3.5)	2.1 (0.00)	12.7 (4.0)	62.5 (9.7)

[a] Mean of duplicate samples.

[b] Standard deviation.

[c] None detected.

Table 3-10. Percentage Distribution of Metabolites at Ten Weeks Post Application in Potato Plants Treated with Aldicarb.

	Aldicarb Ten Weeks				
Compound	Foliage	Stem	Seed	Tuber	Root
T	-[a]	-	-	-	-
T1	41.1[b] (6.00)[c]	24.4 (14.3)	13.7 (7.4)	12.9 (3.2)	2.8 (2.1)
T2	12.4 (0.2)	7.4 (0.4)	-	2.5 (0.2)	1.8 (1.5)
T1 0	2.8 (2.4)	2.5 (2.5)	4.5 (4.5)	8.7 (0.8)	2.6 (1.6)
T1 AM	2.0 (1.0)	2.2 (1.3)	2.6 (2.6)	4.0 (0.6)	1.1 (0.7)
T2 AM	1.5 (0.6)	1.4 (1.4)	-	1.0 (1.0)	0.6 (0.4)
T1 N	2.9 (0.7)	2.9 (2.1)	-	4.1 (0.5)	0.9 (0.7)
T2 N	1.0 (1.0)	0.8 (0.8)	-	-	-
T1 Alc	0.4 (0.7)	-	-	1.8 (0.4)	-
Unknown 1	0.3 (0.1)	-	-	0.8 (0.8)	-
Polar	0.5 (0.01)	0.7 (0.6)	6.1 (4.4)	1.0 (0.1)	1.2 (1.0)
Aqueous	29.7 (11.5)	24.9 (17.9)	21.1 (0.4)	46.5 (11.0)	18.3 (9.3)
Unextractable	5.4 (0.3)	32.8 (7.1)	51.5 (6.00)	16.7 (7.8)	70.7 (17.7)

[a] None detected.

[b] Mean of duplicate samples.

[c] Standard deviation.

Twenty to 28% of the ^{14}C in the foliage, stem and root was aqueous soluble. The seed contained 45% aqueous soluble materials.

At 6 weeks post application only very small amounts of aldicarb were detected in the foliage and seed (0.35% and 1.4%, respectively). Sulfoxide was the major metabolite detected in all plant parts. Tubers were present at 6 weeks and the sulfoxide was the only organic soluble component detected, representing 32.2%. Most of the tuber ^{14}C was aqueous soluble material. Sulfone represented 7.6%, 5.4% and 1.6% of the foliage, stem and seed radiocarbon, respectively.

By 10 weeks post application parent aldicarb was not detected in any plant part. The percentage of radiocarbon represented by the sulfoxide had decreased in all plant parts and there was a slight increase in the percentage of sulfone. In the tubers there was 8.7% sulfoxide oxime, 4.0% sulfoxide amide and 4.1% sulfoxide nitrile. Approximately 10% of the ^{14}C in the foliage, stem and root was distributed among the sulfoxide oxime, sulfoxide and sulfone amide, sulfoxide and sulfone nitrile and sulfoxide alcohol. Almost 50% of the tuber ^{14}C was aqueous soluble material.

The percentage distribution of organic soluble metabolites in the various plant parts of aldicarb sulfoxide treated plants is presented in Tables 3-11, 3-12 and 3-13

Table 3-11. Percentage Distribution of Metabolites at Two Weeks Post Application in Potato Plants Treated with Aldicarb Sulfoxide.

	Sulfoxide Two Weeks				
Compound	Foliage	Stem	Seed	Tuber[a]	Root
T1	57.4[b] (3.00)[c]	45.3 (1.6)	61.8 (2.4)	-	33.2 (0.4)
T2	9.1 (1.0)	5.9 (1.3)	4.3 (1.1)	-	6.9 (0.5)
T1 O	0.3 (0.3)	-	-	-	-
T1 AM	1.5 (1.5)	2.3 (0.7)	1.6 (0.3)	-	6.0 (0.1)
T2 AM	2.4 (0.4)	7.8 (1.6)	2.4 (0.2)	-	-
T1 N	4.7 (0.5)	4.1 (0.5)	2.1 (0.3)	-	5.6 (0.1)
T2 N	0.3 (0.02)	-	0.7 (0.1)	-	1.7 (0.2)
T1 Alc	-[d]	-	0.4 (0.2)	-	-
Unknown 1	-	-	-	-	-
Polar	0.2 (0.2)	0.3 (0.02)	0.2 (0.01)	-	0.6 (0.3)
Aqueous	21.0 (1.9)	29.8 (2.1)	25.0 (8.9)	-	34.1 (0.6)
Unextractable	4.1 (0.2)	4.5 (1.7)	1.5 (0.3)	-	11.9 (0.5)

[a] No tubers at first harvest.

[b] Mean of duplicate samples.

[c] Standard deviation.

[d] None detected.

Table 3-12. Percentage Distribution of Metabolites at Six Weeks Post Application in Potato Plants Treated with Aldicarb Sulfoxide.

	Sulfoxide Six Weeks				
Compound	Foliage	Stem	Seed[a]	Tuber	Root[a]
T1	47.0[b] (5.0)[c]	22.9 (0.5)	7.1	13.7 (0.1)	9.0
T2	12.1 (3.2)	6.1 (0.6)	1.9	0.5 (0.04)	3.8
T1 0	-[d]	-	-	-	-
T1 AM	5.0 (0.3)	4.7 (2.0)	0.8	0.3 (0.1)	3.2
T2 AM	4.5 (1.0)	3.0 (0.8)	3.8	2.0 (0.8)	5.7
T1 N	2.1 (0.5)	1.1 (1.1)	14.5	1.2 (0.9)	3.7
T2 N	-	0.2 (0.2)	-	-	-
T1 Alc	-	-	-	-	-
Unknown 1	-	-	-	-	-
Polar	0.5 (0.5)	-	0.1	-	-
Aqueous	22.4 (3.5)	49.9 (5.7)	53.6	67.0 (8.5)	48.3
Unextractable	6.4 (2.1)	12.1 (1.1)	18.2	15.3 (4.4)	26.3

[a] One sample.

[b] Mean of duplicate samples.

[c] Standard deviation.

[d] None detected.

Table 3-13. Percentage Distribution of Metabolites at Ten Weeks Post Application in Potato Plants Treated with Aldicarb Sulfoxide.

	Sulfoxide Ten Weeks				
Compound	Foliage	Stem	Seed	Tuber	Root
T1	26.8[a] (3.0)[b]	15.9 (0.4)	1.2 (1.2)	16.6 (4.2)	2.3 (0.3)
T2	10.8 (0.9)	6.8 (0.9)	6.5 (5.5)	2.8 (1.0)	1.2 (0.2)
T1 0	4.7 (1.2)	3.2 (0.3)	6.0 (6.0)	10.9 (3.6)	2.1 (0.2)
T1 AM	3.9 (2.8)	2.3 (0.2)	14.7 (1.3)	0.7 (0.7)	1.7 (0.2)
T2 AM	-[c]	-	-	2.2 (1.8)	2.1
T1 N	1.5 (0.1)	1.6 (0.7)	1.5 (0.2)	1.4 (1.4)	0.4 (0.4)
T2 N	1.2 (0.3)	0.7 (0.04)	-	1.4 (1.4)	-
T1 Alc	-	-	-	-	-
Unknown 1	-	-	-	-	-
Polar	0.7	0.5 (0.1)	0.4 (0.1)	1.4 (0.3)	0.8 (0.03)
Aqueous	43.2 (10.2)	40.0 (7.6)	38.5 (5.6)	48.3 (8.0)	41.9 (1.15)
Unextractable	7.2 (4.1)	29.0 (2.2)	31.2 (12.2)	14.3 (3.3)	47.5 (8.1)

[a] Mean of duplicate samples.

[b] Standard deviation.

[c] None detected.

for 2 weeks, 6 weeks and 10 weeks post application, respectively. Reduction of the sulfoxide to aldicarb was not observed in any plant part at any harvest. Therefore, all metabolites and conversion products detected were derived from sulfoxide. At the 2 week interval, parent sulfoxide represented the major percentage of ^{14}C in the foliage, stem, seed and root, 57.6%, 48.7%, 69.3% and 33.7%, respectively. Sulfone and sulfoxide nitrile were the other two major ^{14}C materials present in all plant part extracts. Aqueous soluble components ranged between 20% and 35%.

At 6 weeks post application the percentage of parent sulfoxide had decreased in all plant parts. Most of the reduction in the sulfoxide percentage was accounted for by an increase in aqueous soluble ^{14}C. Tubers were present at 6 weeks and contained 13.7% sulfoxide, 0.50% sulfone and 67.0% aqueous soluble ^{14}C.

By 10 weeks post application, the percentage of parent sulfoxide had further decreased in all plant parts except tubers. In tubers an increase from 13.7% (6 weeks) to 16.6% was observed. The percentage of sulfone increased in tubers to 10.8%, but remained constant or decreased in all other plant parts. Sulfoxide amide and nitrile, and sulfone amide and nitrile were detected in all parts of the plant.

The percentage distribution of organic soluble metabolites in the various plant parts of sulfone treated plants at 2 weeks, 6 weeks and 10 weeks post application are shown in Tables 3-14, 3-15 and 3-16, respectively. Reduction of the sulfone to sulfoxide or to aldicarb was not observed. As a result, the sulfone conversion products were derived directly from aldicarb sulfone. Parent sulfone represented the largest percentage of ^{14}C in all plant parts at 2 weeks post application, 40% to 50% in the stem, seed and root. Almost 72% of the foliage radiocarbon was the sulfone. Most of the remaining radiocarbon in the plant parts was water soluble. The root contained a high unextractable percentage. Other materials identified were sulfone oxime and sulfone amide. There were small percentages of unidentified ^{14}C.

By 6 weeks post application tubers had appeared and contained 28.1% parent sulfone. Most of the tuber ^{14}C was aqueous soluble material. The parent sulfone percentage decreased slightly in all other parts of the plant with concomitant increases in the aqueous component.

At 10 weeks post application 28.2% of the tuber ^{14}C was sulfone with 7.5% as the sulfone oxime and 8.9% as sulfone amide. The percentage of sulfone in the foliage, seed and root remained nearly the same at 6 weeks. The sulfone percentage in the stem decreased.

Table 3-14. Percentage Distribution of Metabolites at Two Weeks Post Application in Potato Plants Treated with Aldicarb Sulfone.

	Sulfone Two Weeks				
Compound	Foliage	Stem	Seed	Tuber[a]	Root
T2	87.5[b] (8.3)[c]	50.9 (10.1)	86.2 (5.6)	-	41.5 (3.2)
T2 0	1.4 (0.1)	4.8 (1.1)	0.7 (0.4)	-	-
T2 AM	0.8 (0.5)	2.5 (1.0)	0.5 (0.1)	-	1.9 (0.9)
T2 N	-[d]	-	-	-	-
Unknown 2	-	-	-	-	-
Unknown 3	0.3 (0.2)	1.4 (0.8)	0.2 (0.02)	-	-
Unknown 4	-	1.9 (1.0)	0.3 (0.3)	-	-
Unknown 5	1.2 (0.6)	2.1 (1.0)	0.5 (0.3)	-	-
Polar	0.2 (0.1)	0.9 (0.2)	0.2 (0.1)	-	2.0 (0.7)
Aqueous	6.4 (0.69)	23.1 (1.4)	1.0 (0.48)	-	14.3 (2.4)
Unextractable	2.2 (0.19)	16.6 (2.7)	0.4 (0.02)	-	40.3 (4.2)

[a] No tubers at first harvest.

[b] Mean of duplicate samples.

[c] Standard deviation.

[d] None detected.

Table 3-15. Percentage Distribution of Metabolites at Six Weeks Post Application in Potato Plants Treated with Aldicarb Sulfone.

	Sulfone Six Weeks				
Compound	Foliage	Stem	Seed	Tuber	Root
T2	62.1[a] (8.3)[b]	33.0 (15.4)	55.0 (0.04)	28.2 (3.4)	13.7 (0.5)
T2 O	4.3 (2.6)	1.4 (0.9)	1.3 (0.4)	3.4 (0.5)	1.1 (1.1)
T2 AM	3.1 (0.8)	2.7 (0.1)	3.3 (0.1)	2.5 (0.6)	7.9 (0.6)
T2 N	1.5 (1.2)	-	0.6 (0.6)	-	-
Unknown 2	-[c]	-	-	-	-
Unknown 3	0.5 (0.2)	0.2 (0.2)	3.1 (0.1)	2.1 (0.1)	-
Unknown 4	-	-	-	-	-
Unknown 5	1.6 (0.3)	1.3 (0.2)	2.4 (0.1)	1.5 (0.4)	-
Polar	0.8 (0.2)	1.4 (0.5)	0.7 (0.03)	0.5 (0.03)	2.0 (0.3)
Aqueous	23.0 (2.5)	41.4 (9.4)	32.1 (0.9)	57.2 (3.7)	33.3 (3.9)
Unextractable	3.1 (0.4)	18.6 (6.1)	1.5 (0.2)	4.6 (1.2)	42.0 (3.6)

[a] Mean of duplicate samples.

[b] Standard deviation.

[c] None detected.

Table 3-16. Percentage Distribution of Metabolites at Ten Weeks Post Application in Potato Plants Treated with Aldicarb Sulfone.

	Sulfone Ten Weeks				
Compound	Foliage	Stem	Seed	Tuber	Root
T2	64.4[a] (1.6)[b]	21.9 (1.7)	48.7 (0.2)	28.1 (2.2)	9.3 (1.1)
T2 O	2.3 (0.3)	3.5 (2.2)	0.2 (0.2)	7.5 (0.7)	1.5 (1.5)
T2 AM	9.9 (4.7)	3.8 (0.6)	7.1 (1.5)	8.9 (2.7)	3.1 (0.2)
T2 N	0.6 (0.03)	-[c]	-	-	-
Unknown 2	0.2 (0.1)	-	-	-	-
Unknown 3	0.6 (0.04)	4.5 (3.7)	5.1 (1.0)	5.0 (0.7)	-
Unknown 4	0.6 (0.1)	-	-	-	-
Unknown 5	1.6 (0.2)	0.6 (0.6)	0.7 (0.7)	4.3 (0.4)	-
Polar	0.8 (0.5)	1.8 (0.6)	1.5 (1.0)	2.3 (0.8)	1.9 (0.7)
Aqueous	16.1 (7.5)	50.3 (5.3)	32.9 (1.1)	41.5 (4.6)	41.8 (0.6)
Unextractable	2.9 (0.2)	13.6 (1.6)	3.8 (0.3)	2.4 (0.2)	42.4 (0.8)

[a] Mean of duplicate samples.

[b] Standard deviation.

[c] None detected.

The pseudo first order rate constants, half-lives and coefficients of determination for parent sulfoxide and parent sulfone in the various potato plant parts are presented in Table 3-17. The rate constants, k, were the slopes of a linear regression from plots of the natural log of parent ppm versus time (days). Half-lives were determined by dividing 0.693 by the rate constants, k and the coefficients of determination represent a measure of the data fit to a linear regression. Parent aldicarb was not detected in any plant part 10 weeks (third harvest) post application; therefore, rate constants could not be calculated. From the data in this experiment, the half-life of aldicarb can only be estimated at less than two weeks. Since parent sulfoxide and sulfone persisted throughout the harvest periods, calculations of rate constants could be performed. The coefficient of determination from tuber samples was 1.0000 because there were only two data points, the second and third harvest. The half-lives of parent sulfoxide and parent sulfone in the foliage were 31.8 days and 36.0 days, respectively, and stem 26.9 days and 27.0 days, respectively. The half-life of sulfone was longer in the tuber than sulfoxide (17.7 days to 8.3 days) and much longer in the roots (59.7 days to 7.0 days).

The analysis of aqueous soluble radiocarbon was a more difficult and a less sensitive procedure than analysis of

Table 3-17. Pseudo First Order Rate Constants, k, Half Lives and Coefficients of Determination for Aldicarb Sulfoxide and Aldicarb Sulfone in Potato Plants.

	Sulfoxide			Sulfone		
	k [a]	$t_{\frac{1}{2}}$ [b]	r^2	k	$t_{\frac{1}{2}}$	r^2
Foliage	0.022	31.8	0.952	0.033	21.0	0.968
Stem	0.026	26.9	0.905	0.026	27.0	0.975
Seed	0.079	8.7	0.999	0.026	27.0	0.975
Tuber[c]	0.084	8.3	1.000	0.039	17.7	1.000
Root	0.099	7.0	0.988	0.012	59.7	0.989
Whole Plant	0.174	27.9	0.850	0.118	24.6	0.990

[a] Pseudo first order rate constant, k, days-1.

[b] Half life, days.

[c] Tubers only at second and third harvest.

organic soluble radiocarbon. Consequently, only data from third harvest samples will be presented and only for foliage and tuber aqueous samples. The analysis consisted of concentration of the aqueous sample followed by ion exchange chromatography. A typical elution pattern from the ion exchange column is shown in Figure 3-1. The sample represented is the third harvest foliage from sulfoxide treated plants. Peak 1, which eluted with the void volume of the column, contained conjugates and peak 2 contained free sulfoxide and sulfone acids. The glycosides were hydrolyzed enzymatically and the aglycones analyzed by TLC. Table 3-18 shows the percentages of aqueous radiocarbon in peak 1 and 2 for aldicarb, aldicarb sulfoxide and aldicarb sulfone treatments at the 10 week harvest. The percentages of ^{14}C in peak 1 of samples from aldicarb and the sulfoxide treated plants were similar, around 80% in the foliage and tuber. The percentage of ^{14}C in peak 1 from samples of aldicarb sulfone treated plants was higher, 92-98%. Peak 1 samples were digested with ß-glucosidase to cleave conjugated species. The percentage of ^{14}C which partitioned into organic solvents after enzymatic digestion is shown in Table 3-19 for the three compounds. In all instances, greater than 65% of the ^{14}C remained water soluble after partitioning. The aqueous soluble ^{14}C was a mixture of the sulfoxide and sulfone acids which had been conjugated. The organic fractions

Figure 3-1. Elution pattern of aqueous radiocarbon on ion exchange column.

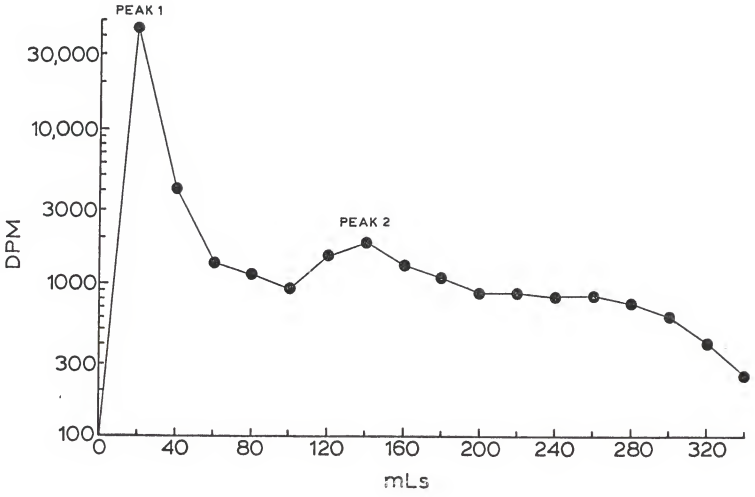

PEAK 1
PEAK 2
30,000
10,000
3000
1000
300
100
DPM
0
40
80
120
160
200
240
280
320
mLs

Table 3-18. Percentage of Radiocarbon in Aqueous Extract in Peak 1 and Peak 2 from Ion Exchange Chromatography of Samples from Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone Treated Plants at the Third Harvest.

	Aldicarb		Sulfoxide		Sulfone	
Plant Part	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
Foliage	79.3	20.7	81.2	18.8	95.6	4.4
Tuber	78.1	21.9	82.1	17.9	98.0	2.0

Table 3-19. Percentage of Radiocarbon in Aqueous and Organic Phases after Enzymatic Digestion of Aqueous Samples from Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone Treated Plants at the Third Harvest.

	Aldicarb		Sulfoxide		Sulfone	
Plant Part	Aqueous	Organic	Aqueous	Organic	Aqueous	Organic
Foliage	74.9	25.1	78.5	21.5	84.7	15.3
Tuber	93.1	6.9	88.0	12.0	65.9	34.1

were concentrated and spotted on TLC for analysis. Table 3-20 shows the percentage distribution of radiocarbon in the organic fractions. Sulfoxide and sulfone alcohol were detected as major aglycones in the foliage and tubers of aldicarb and aldicarb sulfoxide treated plants while the sulfoxide and sulfone amides were only detected in the foliage. Sulfone alcohol was the major aglycone detected in the foliage and tubers of sulfone treated plants and sulfone amide was detected only in the foliage. These data are summarized in Table 3-21 in terms of the percentages of recovered radiocarbon.

Sand radiocarbon. The percentage distribution of organic soluble conversion products, aqueous soluble ^{14}C and unextractable residue (in terms of recovered ^{14}C) in the three sand layers from pots containing plants treated with aldicarb at the three post application harvest periods are shown in Tables 3-22, 3-23 and 3-24. There was very little difference in the percentage distribution of conversion products within a pot for the various layers. Parent aldicarb was detected in the top and middle layers at 2 weeks and 10 weeks. The percentage of parent ranged from approximately 22% at the 2 weeks to approximately 2.5% by 6 weeks and 10 weeks. Sulfoxide represented 30 to 40% of the radiocarbon in the top two layers at two weeks, was present in the bottom layer by 6 weeks and had decreased in all three layers (11.0 to 30.0%) by 10 weeks. The sulfone

Table 3-20. Percentage Distribution of Radiocarbon in Organic Fraction after Enzymatic Digestion of Peak 1 Samples from Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone Treated Plants at the Third Harvest.

	Alidcarb		Sulfoxide		Sulfone	
Compound	Foliage	Tuber	Foliage	Tuber	Foliage	Tuber
T1 AC + T2 AC	10.0	9.3	15.1	6.5	0.7	5.9
T1 AL	30.3	41.0	15.1	32.3	-	-
T1 AM	12.4	-	13.7	-	-	-
T10	-[a]	9.0	5.5	12.9	-	-
T2 AM	15.8	-	14.1	-	14.8	-
T2 AL	29.1	40.7	13.4	48.3	72.0	70.6
Unknown 6	2.4	-	15.5	-	-	-
Unknown 7	-	-	7.6	-	-	-
Unknown 8	-		-	-	12.5	23.5

[a] None detected.

Table 3-21. Percentage Distribution of Aqueous Soluble Metabolites of the Third Harvest in Terms of Recovered Radiocarbon.

	Aldicarb[a]		Sulfoxide		Sulfone	
Compound	Foliage	Tuber	Foliage	Tuber	Foliage	Tuber
T10[b]	-[d]	0.2	0.4	0.6	-	-
T1 AM[b]	0.7	-	1.0	-	-	-
T2 AM[b]	0.9	-	1.1	-	0.4	-
T1 AL[b]	1.8	1.0	1.1	1.5	-	-
T2 AL[b]	1.7	1.0	1.0	2.3	1.6	9.8
T1 AC + T2 AC[c]	6.2	10.2	8.1	8.6	0.7	0.8
T1 AC + T2 AC[b]	18.2	34.1	28.6	35.2	13.1	27.6
Unknown 6[b]	0.1	-	1.2	-	-	-
Unknown 7[b]	-	-	0.5	-	-	-
Unknown 8[b]	-	-	-	-	0.3	3.3

[a] As a percentage of the recovered radiocarbon.

[b] As conjugates.

[c] Free acids.

[d] None detected.

Table 3-22. Percentage Distribution of Conversion Products at Two Weeks Post Application in Sand Layers of Pots Treated with Aldicarb.

	Aldicarb Two Weeks		
Compound	Top[a]	Middle	Bottom
T	23.2 ± 4.3[b]	20.6 ± 4.2	-
T1	38.9 ± 6.6	28.1 ± 3.7	-
T2	4.7 ± 0.2	4.6 ± 0.4	-
T1 0	5.4 ± 2.0	2.7 ± 0.2	-
T1 AM	2.0 ± 1.1	0.7 ± 0.3	-
T2 AM	-[c]	-	-
T1 N	2.3 ± 0.2	0.6 ± 0.03	-
T2 N	0.4 ± 0.1	-	-
T1 Alc	-	-	-
Unknown 1	-	-	-
Polar	-	-	15.6 ± 2.4[d]
Aqueous	13.5 ± 7.7	20.8 ± 4.1	30.1 ± 8.7
Bound	9.6 ± 2.4	21.9 ± 4.5	54.3 ± 6.3

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

[d] Too low a level of 14C for TLC analysis.

Table 3-23. Percentage Distribution of Conversion Products at Six Weeks Post Application in Sand Layers of Pots Treated with Aldicarb.

	Aldicarb Six Weeks		
Compound	Top [a]	Middle	Bottom
T	1.7 ± 0.3[b]	-	-
T1	20.9 ± 3.3	20.1	18.3 ± 5.0
T2	4.1 ± 0.5	6.2	4.8 ± 1.4
T1 0	5.9 ± 0.3	7.3	6.0 ± 1.7
T1 AM	1.5 ± 0.5	3.8	3.2 ± 0.9
T2 AM	-[c]	-	-
T1 N	2.4 ± 0.5	0.4	-
T2 N	-	-	0.6 ± 0.2
T1 Alc	-	-	-
Unknown 1	-	-	-
Polar	-	-	0.1 ± 0.02
Aqueous	39.4 ± 3.9	47.6 ± 2.0	42.7 ± 0.6
Bound	24.1 ± 0.6	14.6 ± 2.0	24.3 ± 9.7

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

[d] Too low a level of 14C for TLC analysis.

Table 3-24. Percentage Distribution of Conversion Products at Ten Weeks Post Application in Sand Layers of Pots Treated with Aldicarb.

	Aldicarb Ten Weeks		
Compound	Top[a]	Middle	Bottom
T	2.3 ± 1.6[b]	3.2 ± 3.2	-
T1	29.1 ± 15.4	22.3 ± 13.7	11.5 ± 2.4
T2	13.7 ± 5.3	10.6 ± 5.8	6.2 ± 1.0
T1 0	2.2 ± 0.8	0.9 ± 0.9	4.4 ± 0.5
T1 AM	1.0 ± 1.0	0.5 ± 0.5	0.9 ± 0.9
T2 AM	-[c]	-	-
T1 N	1.9 ± 0.4	0.5 ± 0.5	-
T1 Alc	-	-	-
Unknown 1	-	-	-
Polar	0.9 ± 0.5	1.1 ± 0.1	2.7 ± 0.9
Aqueous	22.2 ± 3.4	33.8 ± 0.3	50.8 ± 5.4
Bound	26.7 ± 19.6	27.1 ± 13.9	23.7 ± 9.2

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

percentage increased and ranged from 6% to 13% of the radiocarbon by 10 weeks. The percentages of other conversion products, sulfoxide oxime, sulfoxide nitrile, sulfoxide amide and sulfone nitrile were 7% or less throughout the 10 weeks of sampling. Aqueous soluble ^{14}C was 22.4%, 33.8% and 50.8% of the ^{14}C in the top, middle and bottom layers, respectively at 10 weeks. Bound residues were generally between 10% and 30% in all layers at all harvests.

The percentage distribution of conversion products in the sand layers of sulfoxide treated pots are presented in Tables 3-25, 3-26 and 3-27 for 2 weeks, 6 weeks and 10 weeks post application, respectively. There was very little difference in the ^{14}C distribution among sand layers within a pot. The percentage of parent sulfoxide decreased during the 2 to 10 week period in all three layers. Sulfoxide was the major identified residue in all layers at all harvests. The percentage of sulfone was similar in all three layers and ranged between 3.7 and 9.6% at all harvests. Aqueous soluble ^{14}C and bound residues increased over the entire 10 weeks.

The percentage distribution of conversion products in sulfone treated pots is shown in Tables 3-28, 3-29 and 3-30 for 2, 6 and 10 weeks post application, respectively. Again, there was little difference in the ^{14}C distribution among the sand layers within a pot. Parent sulfone

Table 3-25. Percentage Distribution of Conversion Products at Two Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfoxide.

	Sulfoxide Two Weeks		
Compound	Top [a]	Middle	Bottom
T1	48.1 ± 4.5[b]	44.2 ± 2.3	37.0 ± 6.0
T2	7.8 ± 0.6	3.7 ± 1.4	4.8 ± 2.9
T1 0	3.9 ± 0.0	5.3 ± 1.8	4.3 ± 0.6
T1 AM	1.8 ± 0.4	1.6 ± 0.6	1.6 ± 0.1
T2 AM	0.3 ± 0.3	0.5 ± 0.04	0.2 ± 0.2
T1 N	1.1 ± 0.2	1.1 ± 0.4	2.0 ± 0.2
T2 N	0.7 ± 0.5	0.5 ± 0.4	0.8 ± 0.8
T1 Alc	-[c]	-	-
Unknown 1	-	-	-
Polar	0.2 ± 0.1	0.1 ± 0.02	0.3 ± 0.1
Aqueous	20.8 ± 2.8	30.1 ± 2.5	31.7 ± 11.9
Bound	15.3 ± 2.6	12.9 ± 0.9	17.3 ± 4.6

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

Table 3-26. Percentage Distribution of Conversion Products at Six Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfoxide.

	Sulfoxide Six Weeks		
Compound	Top [a]	Middle	Bottom
T1	8.3 ± 0.3[b]	21.0 ± 0.9	24.0 ± 1.3
T2	4.2 ± 0.4	6.4 ± 0.3	6.2 ± 0.2
T1 0	1.7 ± 0.1	7.8 ± 0.6	8.1 ± 0.6
T1 AM	0.6 ± 0.01	4.1 ± 0.3	3.9 ± 0.1
T2 AM	-[c]	-	-
T1 N	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.03
T2 N	-	-	-
T1 Alc	-	-	-
Unknown 1	-	-	-
Polar	-	-	0.1 ± 0.03
Aqueous	73.1 ± 0.3	23.9 ± 1.6	45.5 ± 0.9
Bound	11.7 ± 0.7	36.3 ± 0.5	11.4 ± 1.2

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

Table 3-27. Percentage Distribution of Conversion Products at Ten Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfoxide.

	Sulfoxide Ten Weeks		
Compound	Top [a]	Middle	Bottom
T1	11.7 ± 0.1[b]	19.0 ± 0.9	9.6 ± 0.4
T2	9.0 ± 0.4	9.6 ± 0.1	6.0 ± 0.1
T1 0	5.5 ± 0.4	4.2 ± 0.01	6.4 ± 0.1
T1 AM	4.8 ± 0.03	3.3 ± 0.1	6.0 ± 0.5
T2 AM	-[c]	-	-
T1 N	-	2.3 ± 0.1	1.7 ± 0.1
T2 N	-	2.1 ± 0.1	2.0 ± 0.2
T1 ALC	-	-	-
Unknown 1	-	-	-
Polar	0.6 ± 0.2	0.6 ± 0.1	1.5 ± 0.1
Aqueous	19.1 ± 0.2	22.0 ± 0.6	32.1 ± 0.7
Bound	49.3 ± 0.2	36.9 ± 1.7	34.7 ± 1.8

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

Table 3-28. Percentage Distribution of Conversion Products at Two Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfone.

	Sulfone Two Weeks		
Compound	Top[a]	Middle	Bottom
T2	43.3 ± 5.7[b]	53.1 ± 1.1	56.5 ± 7.5
T2 O	4.8 ± 2.5	3.1 ± 0.7	1.8 ± 1.8
T2 AM	2.8 ± 1.8	0.5 ± 0.2	-
T2 N	-[c]	-	-
Unknown 2	-	-	-
Unknown 3	-	-	-
Unknown 4	-	-	-
Unknown 5	-	2.4 ± 1.2	-
Polar	0.8 ± 0.8	0.04 ± 0.04	0.1 ± 0.1
Aqueous	34.8 ± 12.4	37.5 ± 3.8	25.4 ± 10.2
Bound	13.5 ± 1.7	3.3 ± 0.5	16.2 ± 1.0

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

Table 3-29. Percentage Distribution of Conversion Products at Six Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfone.

	Sulfone Six Weeks		
Compound	Top [a]	Middle	Bottom
T2	39.8 ± 8.0[b]	40.8 ± 1.6	52.1 ± 5.9
T2 O	2.0 ± 0.03	2.9 ± 1.1	6.5 ± 1.2
T2 AM	7.9 ± 1.8	7.2 ± 1.0	9.1 ± 2.6
T2 N	-[c]	-	-
Unknown 2	-	-	-
Unknown 3	-	-	-
Unknown 4	-	-	-
Unknown 5	-	3.3 ± 0.7	0.3 ± 0.3
Polar	0.7 ± 0.1	0.5 ± 0.4	0.5 ± 0.1
Aqueous	8.5 ± 2.1	9.7 ± 2.1	10.9 ± 2.4
Bound	41.1 ± 4.2	35.6 ± 0.6	20.6 ± 1.8

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

Table 3-30. Percentage Distribution of Conversion Products at Ten Weeks Post Application in Sand Layers of Pots Treated with Aldicarb Sulfone.

	Sulfone Ten Weeks		
Compound	Top [a]	Middle	Bottom
T2	26.8 ± 15.4[b]	7.1 ± 2.3	11.7 ± 3.1
T2 O	0.5 ± 0.5	-	-
T2 AM	0.2 ± 0.2	-	-
T2 N	-[c]	-	-
Unknown 2	-	-	-
Unknown 3	-	-	-
Unknown 4	-	-	-
Unknown 5	0.04 ± 0.05	-	-
Polar	-	-	-
Aqueous	7.9 ± 1.5	4.5 ± 0.8	4.6 ± 0.3
Bound	64.5 ± 17.7	88.4 ± 3.1	83.7 ± 2.8

[a] Sand layer.

[b] Mean of duplicate samples and standard deviation.

[c] None detected.

represented the highest percentage of identified ^{14}C and that percentage decreased from 2 weeks to 10 weeks. The percentage of sulfone was similar among the three layers at each of the harvests. Aqueous soluble ^{14}C decreased with time and bound residues increased with time. At 10 weeks bound residues represented 64.5% to 88.4% of the ^{14}C in all layers, which was caused primarily by a decrease in the total dpm content of the sand.

The pseudo first order rate constants, half-lives and coefficients of determination for aldicarb, the sulfoxide and the sulfone in the three sand layers are presented in Table 3-31. These were determined as described above. Also indicated is the ppm in the whole pot. Aldicarb disappeared rapidly and was not detected at the second harvest in the middle layer or at any harvest in the bottom layer. The calculated half-lives in the top and middle layers were 10.5 and 29.7 days, respectively. The half-lives in the top, middle and bottom layers of the sulfoxide treated pots were 11.5, 14.3 and 13.8 days, respectively. Sulfone had a higher half-life in the top layer (26.6 days) than sulfoxide, but in the bottom two layers (8.0 and 17.3 days) the half-lives were similar to the sulfoxide.

Discussion

The absorption of radiolabeled aldicarb and aldicarb sulfoxide by potato plants was more rapid than the absorption of radiolabeled aldicarb sulfone. At two weeks

Table 3-31. Pseudo First Order Rate Constants, k, Half Lives and Coefficients of Determination for Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone in Sand Layers from Pots Containing Treated Potato Plants.

	Aldicarb			Sulfoxide			Sulfone		
	k [a]	$t_{\frac{1}{2}}$ [b]	r^2	k	$t_{\frac{1}{2}}$	r^2	k	$t_{\frac{1}{2}}$	r^2
Top	0.066	10.5	0.676	0.060	11.5	0.997	0.026	26.6	0.974
Middle	0.023[c]	29.7	1.000	0.049	14.3	0.919	0.087	8.0	0.998
Bottom	-[d]	-	-	0.050	13.8	0.906	0.040	17.3	0.776
Whole Pot	0.456	10.6	0.750	0.365	13.3	0.999	0.298	16.3	0.990

[a] Pseudo first order rate constant, k, days.

[b] Half life, days.

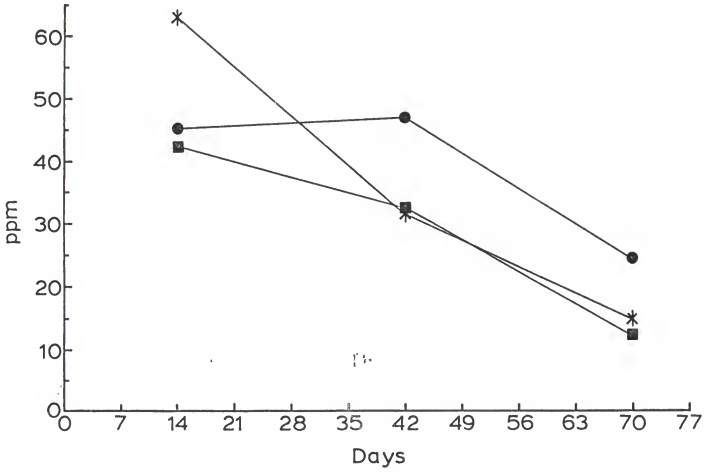
[c] Aldicarb detected only at first and third harvest.

[d] None detected at any harvest.

post application 40% to 50% of the applied radiocarbon was in the plant when aldicarb or sulfoxide was applied. With all three compounds the percentage of the applied ^{14}C in the plant remained the same throughout the three harvests. This is supported by the results of an analysis of variance of the percentage means for each experiment using a completely randomized design. Sulfone was absorbed at a somewhat slower rate than either aldicarb or the sulfoxide and did not reach as high a percentage of the applied ^{14}C. The percentage of applied radiocarbon in the sulfone treated plants ranged between 24 and 38% throughout the harvest period.

Evaluation of these percentages of the applied ^{14}C (Table 3-3) indicates that the actual radiochemical content (in terms of dpm) of the aldicarb and sulfoxide treated plants either remained the same or increased slightly throughout the three harvests. The sulfone treated plants increased in total radiocarbon content from 2 weeks to 6 weeks and then decreased from the 6 week harvest to 10 weeks post application. When considering the data in terms of ppm in the whole plant (Table 3-5 and Figure 3-2) there was a decrease from 2 weeks to 10 weeks for all three treatments (aldicarb, sulfoxide and sulfone). This decrease occurred as a result of plant growth and an increase in total plant weight as time post application increased (Table 3-2). Therefore, even though the total

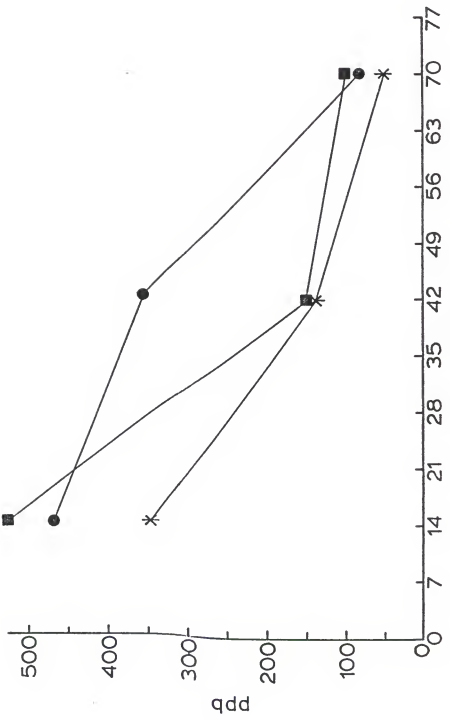
Figure 3-2. ppm as radiocarbon equivalents in whole potato plants treated with aldicarb (*), sulfoxide (●) and sulfone (■).


60
50
40
30
20
10
0
ppm
0
7
14
21
28
35
42
49
56
63
70
77
Days

radiocarbon content of the whole plant remained the same throughout the sampling period, there was a decrease in radiocarbon content on a unit per weight basis.

The percentage of the applied ^{14}C in the sand (whole pot) decreased with time for all three compounds (Table 3-3). There was also a decrease in the ppb with time (Table 3-7 and Figure 3-3). The reduction of radiocarbon in the sand probably resulted from a combination of three factors, uptake by the plant, presence in drainage water from the bottom of the pot and volatilization from the sand surface. Uptake of ^{14}C by the plant was the major source of depletion, particularly at the 2 week harvest. At the 6 week and 10 week harvests there was not a large enough increase in the plant ^{14}C of aldicarb and sulfoxide treated plants to account for the decrease in sand ^{14}C. A portion of these reductions at 6 weeks and 10 weeks could be accounted for by plant uptake if plant radiocarbon had been volatilized from plant surfaces as either ^{14}C organic compounds or $^{14}CO_2$. This process would allow the plant to continue uptake of ^{14}C residues while the total plant ^{14}C content would remain the same. At 6 weeks post application in the sulfone application, plant uptake accounted for most of the reduction in sand radiocarbon. Losses through the drainage water from the pots was probably a factor at all harvests for all treatments. Losses by volatilization from the sand

Figure 3-3. ppb as radiocarbon equivalents in sand from pots treated with alicarb (*), sulfoxide (●) and sulfone (■).


500
400
300
200
100
0
ppb
0
7
14
21
28
35
42
49
56
63
70
77
Days

surface could have been in the form of volatilized ^{14}C organic compounds or $^{14}CO_2$ produced by microbial degradation near the surface. Previous experiments in growth chambers with plants treated with ^{14}C dieldrin indicated that cross contamination of control (nontreated) plants by volatilized dieldrin was significant (Stratton and Wheeler, 1983). In the present experiment, analysis of control plants maintained in the same growth chambers as treated plants showed no significant radiocarbon residues.

The total recovered radiocarbon as a percentage of the applied was above 50% for all three compounds at all harvest periods (Table 3-3) except the sulfone third harvest (32.4%). The percentages were generally higher at the early harvests.

Distribution of Radiocarbon Residue within the Plant

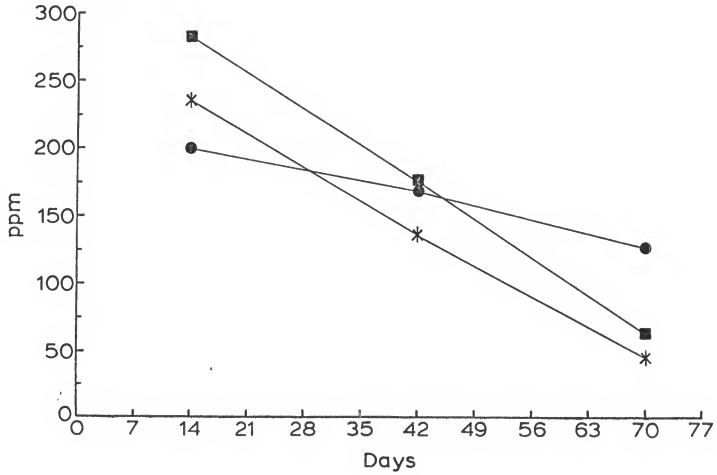
Once the radiocarbon was taken up by the plant from the soil it was rapidly translocated to the foliage. This occurred for all three compounds, aldicarb, aldicarb sulfoxide and aldicarb sulfone. Of the recovered ^{14}C in the plant, 70% to 83% of it was in the foliage by 2 weeks post application and the percentage had increased to 87% to 93% by 10 weeks post application for all three compounds. Ninety-three to 95% of the radiocarbon in the plant at 10 weeks was in the leaves and stems.

The percentage of plant ^{14}C in the seed potato was relatively high at the first harvest since the test

compounds were placed directly on the seed. By 10 weeks, 1.0% or less of the ^{14}C was in the seed. Tubers, which had appeared by 6 weeks, showed an increase in the percentage of plant ^{14}C from 6 weeks to 10 weeks. At 10 weeks post application the percentage distribution of ^{14}C in plants treated with aldicarb sulfoxide and sulfone was foliage >> stem $\geq$ tuber $\geq$ seed $\geq$ root.

When the total residue content is considered in terms of ppm (as ^{14}C equivalents) the values were quite high for some of the plant parts. The ppm levels in the foliage at all harvest periods for the three compounds are shown in Figure 3-4. The ppm levels, as ^{14}C equivalents, in the other plant parts are in Figures 3-5, 3-6 and 3-7, for aldicarb, aldicarb sulfoxide and aldicarb sulfone treated plants, respectively. Foliage ppm levels were particularly high; at 10 weeks they were 44.6, 125.9 and 62.1 for aldicarb, the sulfoxide and the sulfone treated plants, respectively (Table 3-5). Compared to literature values of ppm levels in the foliage of potato plants (Andrawes et al., 1971) these levels are quite high. In a radiochemical experiment with aldicarb in field grown potato plants Andrawes et al. (1971) found 4.38 ppm at 90 days in the foliage (leaves and stem) which represented 2.04% of the applied ^{14}C. This is considerably lower than the ppm levels for aldicarb (44.6), sulfoxide (125.9) and sulfone (62.1) and percentage of applied ^{14}C (21.7% for sulfone and

Figure 3-4. ppm of total ^{14}C residues of aldicarb (*), sulfoxide (●) and sulfone (■) as radiocarbon equivalents in potato plant foliage.


300
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150
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50
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ppm
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14
21
28
35
42
49
56
63
70
77
Days

Figure 3-5. ppm of total ^{14}C residues of aldicarb as radiocarbon equivalents in potato plants stem (*), seed (●), tuber (■) and root (▲).

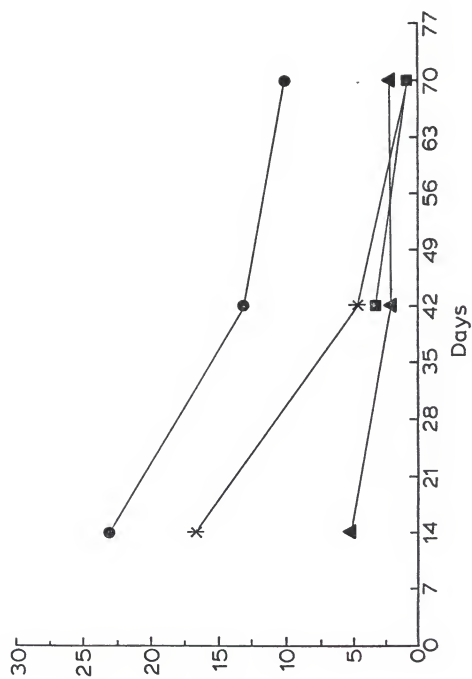

30
25
20
15
10
5
0
ppm
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-6. ppm of total ^{14}C residues of sulfoxide as radiocarbon equivalents in potato plant stem (*), seed (●), tuber (■) and root (▲).

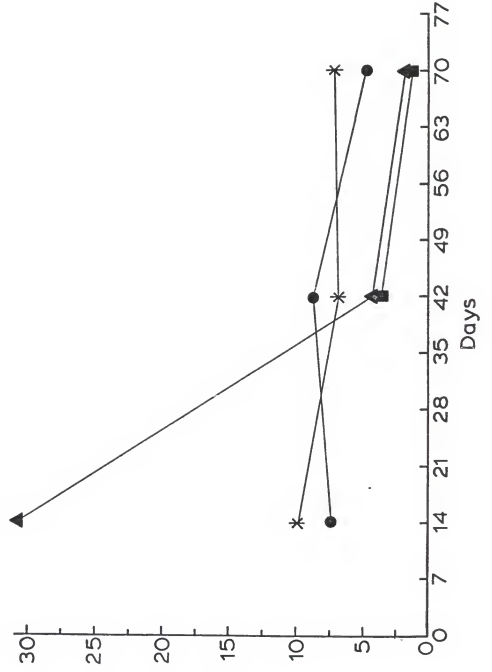
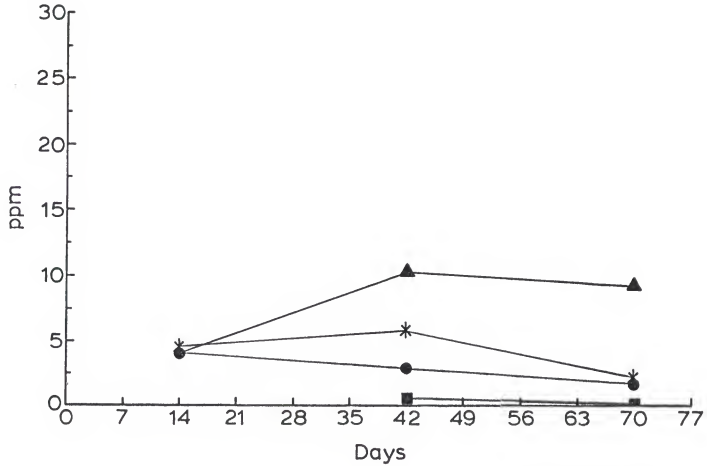

ppm
30
25
20
15
10
5
0
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-7. ppm of total ^{14}C residues of sulfone as radiocarbon equivalents in potato plant stem (*), seed (●), tuber (■) and root (▲).


30
25
20
15
10
5
0
ppm
0
7
14
21
28
35
42
49
56
63
70
77
Days

46.7% for aldicarb and sulfoxide) found in the current experiment at 70 days. To make a direct comparison with these results the foliage and stem data in the present experiment must be combined since the literature data for foliage includes the stem. This is a significant adjustment since the total stem weights were generally about equal to the total foliage weights (Table 3-2) and the stem ppm were approximately 1/10th to 1/30th of the foliage ppm. Therefore the adjusted foliage (foliage plus stem) ppm were 22.7, 66.5 and 32.2 for aldicarb, the sulfoxide and the sulfone treated plants at 10 weeks, still higher than the results of Andrawes et al. (1971). Their results from field grown potatoes should be similar to those found under normal agricultural conditions. In the present case, however, the application rate was calculated based on the surface area of the five gallon pot in which the potatoes were grown. Upon application the full 3.36 kg/ha rate of formulated granules were placed in direct contact with the seed potato. Then the radiolabeled compound was pipetted directly onto the seed. This procedure greatly enhanced the effective application rate and uptake. During normal agricultural applications the granules are buried on the sides of mounds containing seed potatoes where only small amounts of granules come in contact with the seed. Therefore, it should not be

surprising to find elevated ppm and percentages of applied levels from a somewhat artificial application procedure.

The ppm levels, as ^{14}C equivalents, found in the tubers of plants treated with aldicarb, aldicarb sulfoxide and aldicarb sulfone were 0.79, 1.20 and 0.18, respectively, at 10 weeks post application. The 0.79 ppm level in tubers from the aldicarb experiment was the same as the 0.79 ppm level of aldicarb equivalents reported by Andrawes et al. (1971) in tubers from field grown potato plants. The ppm level in the seed piece in their experiment decreased as time post application increased, as was the case in the present experiment.

Parent Distribution

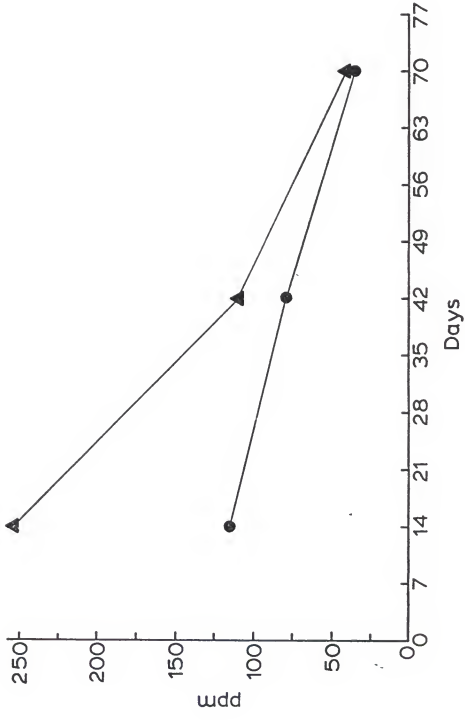
The three parent chemicals, aldicarb, aldicarb sulfoxide and aldicarb sulfone, were all biologically unstable under the experimental conditions. Extensive degradation occurred in all plant parts and sand layers. There were, however, some quantitative differences in the extent of metabolism of the three compounds in the various plant parts. Within the sand there were less pronounced differences in the parent degradation among the three sand layers.

The disappearance of parent aldicarb was very rapid in all plant parts. None was detected in any part of the plant at 10 weeks post application. Parent aldicarb was detected in the foliage, stem and seed at two weeks post

application, at 5.49 ppm, 1.0 ppm and 6.13 ppm, respectively, and the foliage and seed at 6 weeks post application, at levels of 0.47 ppm and 0.18 ppm, respectively. A half-life in the whole plant for aldicarb can only be estimated to be less than 2 weeks since parent aldicarb represented less than 25% of the ^{14}C at 2 weeks and had virtually disappeared by 6 weeks. Aldicarb was detected in the top layer of sand at all three harvests and the levels decreased with time. There was 223.6 ppb at 2 weeks, 3.8 ppb at 6 weeks and 5.5 ppb at 10 weeks. Small amounts were detected in the middle layer at the first and third harvests, 12.9 ppb and 3.5 ppb, respectively. None was detected in the bottom layer at any harvest.

The situation regarding the presence of parent compound was somewhat different in the sulfoxide and sulfone treated plants. Unlike aldicarb, both parent sulfoxide and parent sulfone were detected at all harvest periods and in all plant parts. The ppm levels of the parent compounds were approximately an order of magnitude greater in the foliage than in the other plant parts in both sulfoxide and sulfone treated plants. Figure 3-8 illustrates the ppm levels vs time. The ppm levels of parent sulfoxide and parent sulfone at 2 weeks were 116 ppm and 253 ppm and at 10 weeks approximately 36 ppm. Both compounds decreased in concentration with time. The parent levels in the other plant parts, stem, seed, tuber and root

Figure 3-8. Parent sulfoxide (●) and parent sulfone (▲) ppm in potato plant foliage.


250
200
150
100
50
0
ppm
0
7
14
21
28
35
42
49
56
63
70
77
Days

were much lower than in the foliage and are shown in Figures 3-9 and 3-10. The level of parent sulfoxide decreased rapidly from 2 weeks to 6 weeks in all plant parts. Parent sulfone, on the other hand, exhibited a small decrease from 2 weeks to 6 weeks in the stem and root and a more rapid decrease in the seed. By 10 weeks there was an overall decrease in the parent sulfone concentration for all plant parts. This slower reduction in sulfone concentration corresponds to the increased uptake of ^{14}C residues by the plant at 6 weeks (Tables 3-3).

The ppb levels of parent sulfoxide and parent sulfone in the three sand layers are shown in Figures 3-11 and 3-12, respectively. At 2 weeks there was a difference in the ppb level in the three layers for both compounds. In the sulfoxide treated pots the order in decreasing ppb was top > middle > bottom and in sulfone treated pots middle > top > bottom. This indicates a more immediate movement of sulfone from the top layer to the middle layer. By 6 weeks and 10 weeks there was little difference in the parent ppb levels among the three layers and between the two compounds.

Metabolites

The array of metabolic products was qualitatively similar among the various plant parts within a given experiment and is illustrated in Figure 3-13 for aldicarb and sulfoxide and Figure 3-14 for sulfone.

Figure 3-9. Parent sulfoxide ppm in potato plant stem (*), seed (●), tuber (■) and root (▲).

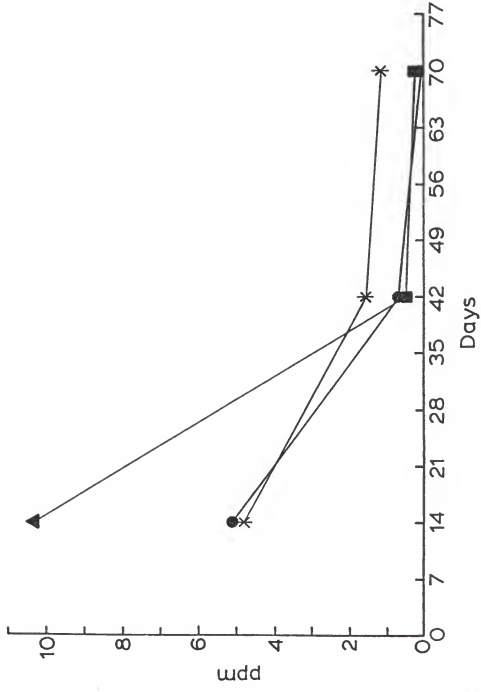

ppm
0
2
4
6
8
10
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-10. Parent sulfone ppm in potato plant stem (*), seed (●), tuber (■) and root (▲).

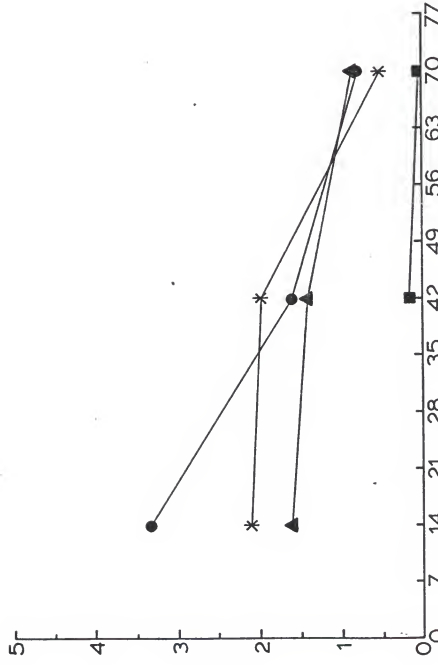

ppm
0
1
2
3
4
5
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-11. Parent sulfoxide ppm in top (*), middle (●) and bottom (■) sand layers.

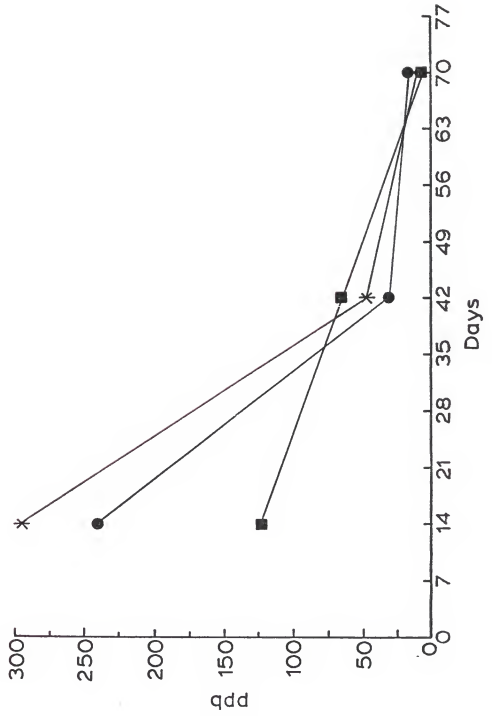

300
250
200
150
100
50
0
ppb
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-12. Parent sulfone ppb in top (*), middle (●) and bottom (■) sand layers.

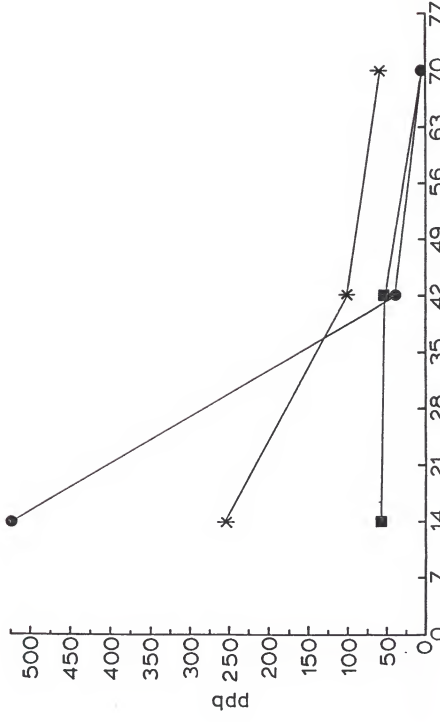

ppb
500
450
400
350
300
250
200
150
100
50
0
0
7
14
21
28
35
42
49
56
63
70
77
Days

Figure 3-13. Proposed metabolic pathway of aldicarb and aldicarb sulfoxide in potato plants.

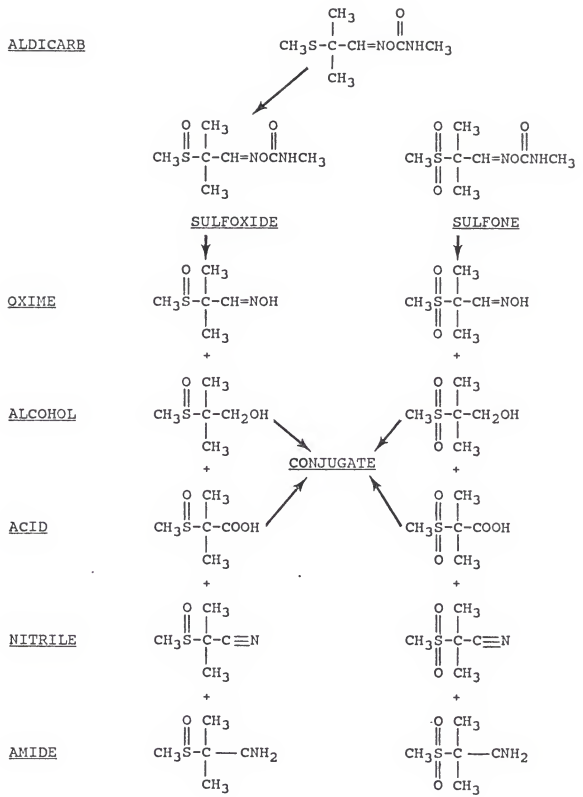
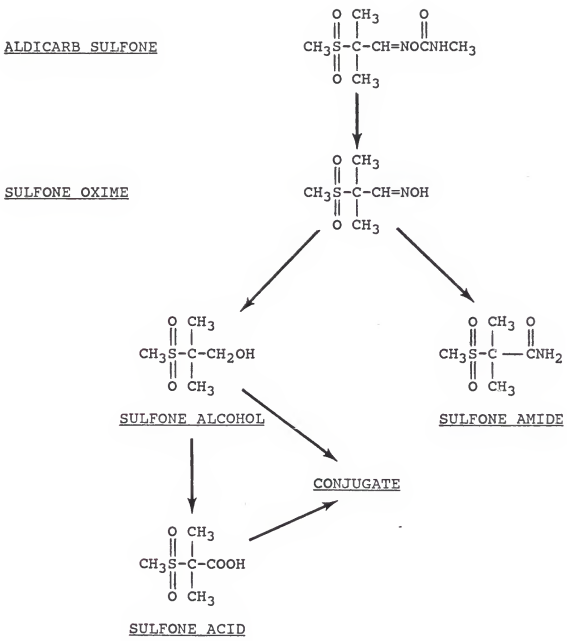

ALDICARB
SULFOXIDE
SULFONE
OXIME
ALCOHOL
CONJUGATE
ACID
NITRILE
AMIDE

Figure 3-14. Proposed metabolic pathway of aldicarb sulfone in potato plants.


ALDICARB SULFONE
SULFONE OXIME
SULFONE ALCOHOL
SULFONE AMIDE
CONJUGATE
SULFONE ACID

In the aldicarb experiment the metabolic products were qualitatively similar in all the plant parts by 10 weeks post application. The differences were primarily in the quantitative distribution of the metabolic products. Generally, there were more oxidative reaction products in the foliage than in the below ground parts of the plant. Also, there were generally more hydrolytic reactions occurring in the tubers than in the foliage as evidenced by a larger percentage of water soluble materials at the later harvests in the tubers.

The metabolite percentage distribution in aldicarb treated plants and sulfoxide treated plants at 10 weeks post application was similar (Tables 3-10 and 3-13). In each there was a higher percentage of sulfone (10% to 12%) in the foliage than in the tubers (2.5% to 2.8%) and a higher percentage of sulfoxide oxime (8.7% to 10.9%) in the tubers than in the foliage (2.5% to 2.8%). In addition, there was a higher percentage of aqueous soluble radiocarbon in the tubers than in the foliage. The aqueous radiocarbon consisted of conjugates of sulfoxide oxime, sulfoxide and sulfone alcohol, sulfoxide and sulfone amides and sulfoxide and sulfone acids. The free acids were also present in the aqueous phase. There was no evidence of a reduction of sulfoxide back to aldicarb.

In the sulfone treated potato plants there were also more hydrolytic reactions occurring in the tubers than in

the foliage. At 10 weeks post application the percentage of parent sulfone in the tuber was 28.3% (approximately 0.1 ppm) compared to 64.2% (approximately 40 ppm) in the foliage indicating that the parent was less stable in the tubers. Also, the percentage of aqueous soluble radiocarbon was much higher in the tubers (41.5%) than in the foliage (16.1%). Again, the aqueous soluble radiocarbon consisted primarily of conjugates of sulfone acid, sulfone alcohol and sulfone amide. There was an unknown conjugate, termed unknown 8, which was tentatively identified as demethyl sulfone. There was also a small amount of free acid.

The conversion products found in the sand were qualitatively similar to metabolites found in the various plant parts (see Figures 3-13 and 3-14). Generally, for all three compounds there was little difference in the percentage distribution among the three sand layers within a pot. There was also a general decrease in the parent percentage and concentration as time increased (see Tables 3-22 to 3-30 and Figures 3-11 and 3-12). In the aldicarb experiment reductions in the percentage of aldicarb corresponded to increases in the percentage of sulfoxide. In the sulfoxide and sulfone experiments the percentage of parent sulfoxide and sulfone decreased with time although in most instances the parent compound remained the major identified component. Percentages of aqueous soluble and

unextractable residue increased with time. The actual total ^{14}C decreased over the 10 week period (Table 3-3 and Figure 3-3). This particular phenomenon can be used to explain high percentages of unextractable material at the later harvests. For example, the total dpm unextracted in the top sand layer of sulfone treated plants at the first harvest was 530500 dpm and the percentage unextracted was 13.45%. At the third sulfone harvest in the top, middle and bottom layers there was 738100 dpm, 346400 dpm and 364600 dpm, which represented 64.5%, 88.4% and 83.7% unextractable residue, respectively. Therefore, the actual quantity of unextractable material remained relatively constant throughout the harvests. This may suggest that sites available for "binding" were occupied early and did not increase. Consequently, increases in percentage bound resulted from reductions in the total actual dpm in the sand layer.

Percentages of sulfone in the potato plants resulting from application of either aldicarb or aldicarb sulfoxide were similar, approximately 11.0% in the foliage and 2.5% in the tubers at 70 days post application. These values were significantly higher than percentages of sulfone found in aldicarb treated potato plants by Andrawes et al. (1971). They found 55.9% sulfone in the foliage and 10.1% in the tubers at 90 days post application and concluded that oxidative metabolism of aldicarb was more active in

the foliage than in the tubers. However, they stem injected potato plants and found smaller percentages of sulfone (1.0 to 3.7% sulfone post injection). The differences in the above results could be a result of varietal differences in Solanum tuberosum, however, there is another possible explanation which concerns the rate of aldicarb conversion to sulfone in the soil.

The stability of aldicarb in soils is a function of a number of variables such as soil type, percentage water, pH, microbial activity and temperature and can have a large effect on the rate and extent of conversion of aldicarb and aldicarb sulfoxide to the sulfone (Smelt et al., 1978a, 1978b, and 1978c, Bull et al., 1970, and Bromilow et al., 1980). Bull et al. (1970) found very little conversion of aldicarb to sulfone (approximately 7.1%) in construction sand at 28 days post application. This result compares favorably with the results obtained in the aldicarb and sulfoxide treated sand from the present experiment. In contrast, Bull et al. (1970) found in a Lufkin fine sandy loam 33.4% sulfone 28 days after treatment with aldicarb.

It is possible then that the percentage of sulfone present in plants grown in soil could be influenced by the concentration of sulfone in the soil. The array of metabolites found in aldicarb treated soils is similar to that found in plants (Coppedge et al., 1967, Bull et al., 1968, Bull et al., 1970, Andrawes et al, 1971b). Many of

these soil metabolites can then be absorbed and translocated by the plant, a situation which makes it difficult to differentiate metabolites produced by the plant from those produced in the soil (Ridgeway et al., 1968). Andrawes et al. (1971) did not report data on the residue content of soils in which their plants were grown, therefore, it is difficult to assess the contribution of soil produced sulfone to the levels found in their plants.

This phenomenon does not only occur with aldicarb in potato plants, but can occur with any appropriate xenobiotic in any plants grown in a metabolically active substrate. Data from experiments with plants grown in different soil types are important to provide information on the overall residue content in plants obtained from the application of the chemical under "normal" environmental conditions. This allows a better estimate of the type of behavior to expect from a chemical in "real world" situations. However, it is difficult from these types of experiments to determine the actual metabolic capability of the plant. In order to do this the plants must be separated from external environmental factors and controlled conditions must be maintained. Some possible approaches include in vitro techniques using both tissue culture, cell free extracts and purified enzyme preparations. Of course, these experiments also have limitations which can affect the interpretation of results.

CHAPTER IV

METABOLISM OF ALDICARB, ALDICARB SULFOXIDE AND ALDICARB SULFONE IN POTATO TUBER AND CITRON CALLUS TISSUE CULTURES

Materials and Methods

Chemicals

Radiochemicals. S-methyl [^{14}C] aldicarb (specific activity 6.06 mCi/mM) and S-methyl [^{14}C] aldicarb sulfone (specific activity 7.1 mCi/mM) were gifts of Union Carbide Corporation. S-methyl [^{14}C] aldicarb sulfoxide was prepared from S-methyl [^{14}C] aldicarb by peracetic acid oxidation (Payne et al., 1966). The reaction was performed by stepwise addition of peracetic acid to an equimolar solution of [^{14}C] aldicarb in ethyl acetate (32-37°C) over a 60 minute period. The progress of the reaction was followed by TLC. Upon conversion of the majority of material to the sulfoxide, water was added and the mixture was partitioned four times with chloroform. The chloroform fractions were combined and concentrated. The [^{14}C] aldicarb, [^{14}C] aldicarb sulfoxide and [^{14}C] aldicarb sulfone were purified by TLC.

Analytical standards. Analytical reference standards furnished by Union Carbide Corporation were: aldicarb (T), aldicarb sulfoxide (T_1), aldicarb sulfone (T_2), aldicarb sulfoxide oxime (T_1O), aldicarb sulfone oxime (T_2O), aldicarb sulfoxide nitrile (T_1N) and aldicarb sulfone nitrile (T_2N).

Most solvents used were Fisherbrand pesticide grade. In some cases technical grade chloroform was subjected twice to fractional distillation. All water used had been deionized and carbon filtered.

Tissue culture media. The medium used was that of Murashige and Skoog (Murashige and Skoog, 1962); its composition was as follows: NH_4NO_3 1650 mg/L, KNO_3 1900 ml/L, H_3BO_3 6.2 mg/L, KH_2PO_4 170 mg/L, KI 0.83 mg/L, $NaMoO_4$-$2H_2O$ 0.25 mg/L, $CoCl_2$-$6H_2O$ 0.024 mg/L, $CaCl_2$-$2H_2O$ 440 mg/L, $MgSO_4$-$7H_2O$ 370 mg/L, $MnSO_4$-H_2O 16.9 mg/L, $ZnSO_2$-$7H_2O$ 10.6 mg/L, $CuSO_4$-$5H_2O$ 0.024 mg/L, Na_2EDTA 37.3 mg/L, $FeSO_4$-$7H_2O$ 27.8 mg/L, myo-inositol 100 mg/L. The concentrations of vitamin, organic and hormone constituents were: thiamine 2.0 mg/L, nicotinic acid 1.0 mg/L, pyridoxine HCl 2.0 mg/L, glycine 0.4 mg/L, sucrose 30 g/L, kinetin 0.01 mg/L, gibberellic acid 0.75 mg/L, 2,4-dichloro-phenoxy acetic acid 10.0 mg/L. The same constituent concentrations were used for both initiation of callus formulation and maintenance of callus cultures.

The pH was adjusted to 5.4-5.6 and agar was added (9 g/L of Difco Bacto-Agar).

The medium for the citron callus tissue experiment was similar in composition. The only differences were as follows: sucrose 50 g/L, 2,4-dichlorophenoxy acetic acid 1.0 mg/L and there was no kinetin or gibberellic acid. The pH was 5.7-5.8. The same growth medium was used for initiation and maintenance of the citron callus cultures.

The media were prepared using deionized, carbon filtered water. The pH was adjusted using 1.0 M HCl or 1.0 M NaOH. Fifty mL of the agar media were dispensed into either 125 mL Erlenmeyer flasks or 250 mL Erlenmeyer flasks, plugged with foam plugs and steam sterilized by autoclaving at 121°C and 103 kPa for 25 minutes.

Initiation and Maintenance of Callus Cultures

Tuber callus tissue was prepared from Atlantic variety potatoes (Solanum tuberosum). Unpeeled potato tubers were surface sterilized by immersion in an aqueous solution of approximately 1.0% v/v sodium hypochlorite for 10 minutes. Using aseptic technique, 15 mm cylindrical cores were taken through the long axis of the tuber with a cork borer. Approximately 5 mm of each end of the core was removed with a scalpel. The cylinder was cored again with a 10 mm cork borer and 5 mm of each end removed. The cylinder of tuber tissue was then sliced into a 2-4 mm disks. The disks were placed on the solid agar medium in 250 mL Erlenmeyer

flasks. Callus tissue which had formed on the disks by 3-4 weeks was removed and transferred to flasks containing fresh agar medium. Subculturing of tissue to fresh agar medium was done every 3-4 weeks.

Citron callus tissue was prepared from undeveloped seed ovules from an Arizona cultivar of the citrus species of citron (Citrus medica). The ovules are located in the center of the fruit and are simply seed pouches without seeds. Ovules were isolated and sterilized by irrigating them with aqueous solutions of 1.0% hypochlorite followed by sterile water. The ovules were placed on the citron solid agar medium for initiation of callus tissue growth. Subculturing of the callus tissue was done every 3-4 weeks.

Chromatographic and Radiochemical Procedures

Thin layer chromatography. TLC was done on silica gel 60 F-254 chromatoplates (0.25 mm X 20 cm, E. Merck) utilizing the following solvent systems: (A) chloroform:n-hexane:ethyl acetate:ethanol (5:1:1:1); (B) methylene chloride:acetonitrile (3:1); (C) chloroform:methanol(6:1); (D) diethylether:n-hexane: ethanol (4:1:1); (E) benzene:methanol:g-acetic acid (4:1:1). Systems A and B were used for analysis of all organic phase extracts for percentage distribution of ^{14}C components. Systems C, D and E, as well as A and C, were used for qualitative identifications. The chemical names, common names, abbreviations and TLC R_f values for aldicarb

and related compounds for the indicated solvent systems are shown in Table 4-1.

Mass spectrometry. Mass spectra of reference standards were recorded on Finnigan 4021 Gas Chromatograph/Mass Spectrometer (GC/MS) operated in the electron impact (EI) mode. Pure samples of T, T1, T10, T2, T20 and T2N were analyzed by direct inlet probe. Probe temperature was increased from 40°C to 350°C until volatilization of the compounds occurred. Source temperature was 250°C, the emission current was 0.35 mA and electron energy was 70 eV. A sample of T1N was analyzed by GC/MS on a 15 m X 0.32 mm DB-5 capillary column (J & W), temperature programmed from 50°C to 200°C at 10 C/min. The injection port temperature was 200°C and other MS conditions were as described above.

The identity of the reference standards was confirmed by EI/MS as described above. The spectra from these analyses are shown in the Appendix (Figures A-1, A-2, A-3, A-4, A-5, A-6, A-7 and A-8). The T, T1, T10 and T20 spectra matched reference spectra in the on line National Bureau of Standards library in the Finnigan 4021 GC/MS/DS and were similar to published EI and CI spectra (Benson and Damico, 1968, Muszkat and Aharanson, 1982 and Wright et al, 1982). The library chose T20 to match with the spectrum for T2, however, comparison of the T2 spectrum with the library T2 spectrum showed similar major ions. Reference

Table 4-1. Thin-Layer Chromatographic Behavior of Aldicarb and Related Standards.

Compound(Common Name)	Abbreviation	Rf Value in Indicated Solvent System[a]				
		A	B	C	D	E
2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb)	T	0.92	0.85	0.95	0.98	0.85
2-Methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb sulfoxide)	T1	0.45	0.10	0.68	0.24	0.89
2-Methyl-2-(methylsulfonyl)propionaldehyde O-(methylcarbamoyl)oxime(Aldicarb sulfone)	T2	0.61	0.61	0.79	0.59	0.55
2-Methyl-2-(methylsulfinyl)propionaldehyde oxime(Sulfoxide oxime)	T1 O	0.52	0.14	0.59	0.73	0.67
2-Methyl-2-(methylsulfonyl)propionaldehyde oxime(Sulfone oxime)	T2 O	0.73	0.74	0.75	0.95	0.73
2-Methyl-2-(methylsulfinyl)propionitrile (Sulfoxide nitrile)	T1 N	0.68	0.74	0.85	0.64	0.62
2-Methyl-2-(methylsulfonyl)propionitrile (Sulfone nitrile)	T2 N	0.85	0.67	0.92	0.88	0.70
2-Methyl-2-(methylsulfinyl)propionamide (Sulfoxide amide)	T1 AM	0.36	0.09	0.60	-	-
2-Methyl-2-(methylsulfonyl)propionamide (Sulfone amide)	T2 AM	0.53	0.35	0.71	-	-
2-Methyl-2-(methylsulfinyl)propanol (Sulfoxide alcohol)	T1 AL	0.23	0.07	0.49	-	-
2-Methyl-2-(methylsulfonyl)propanol (Sulfone alcohol)	T2 AL	0.63	0.61	0.65	-	-
2-Methyl-2-(methylsulfinyl)propionaldehyde O-carbamoyloxime(Demethyl sulfoxide)	DT1	-	0.08[b]	0.47[b]	0.12[c]	-
2-Methyl-2-(methylsulfonyl)propionaldehyde O-carbamoyloxime(Demethyl sulfone)	DT2	-	0.41	0.58[b]	0.45[c]	-
2-Methyl-2-(methylthio)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT	-	0.55[b]	0.78[b]	-	-
2-Methyl-2-(methylsulfinyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT1	-	0.00[b]	0.42[b]	-	-
2-Methyl-2-(methylsulfonyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT2	-	0.29[b]	0.62[b]	-	-
2-Methyl-2-(methylsulfinyl)propionic acid (Sulfoxide acid)	T1 AC	-	0.00	Streaked[b]	-	0.70[b]
2-Methyl-2-(methylsulfonyl)propionic acid (Sulfone acid)	T2 AC	-	0.00	Streaked[b]	-	0.72[b]
N-hydroxy-2-methyl-2-(methylsulfinyl)-propionamide	T1 HA	-	0.00[b]	Streaked[b]	-	0.29[b]
N-hydroxy-2-methyl-2-(methylsulfonyl)-propionamide	T2 HA	-	0.00[b]	Streaked[b]	-	0.51[b]

[a] A, chloroform:hexane:ethyl acetate:ethanol (5:1:1:1); B, methylene chloride:acetonitrile (3:1); C, chloroform:methanol (6:1); D, diethyl ether:n-hexane:ethanol (4:1:1); E, benzene:methanol: acetic acid (4:1:1).

[b] From Bartley et al., 1970.

[c] From Coppedge et al., 1967.

spectra for T1N and T2N were not in the library, however, they show characteristic nitrile fragmentation.

Radiochemical analysis. All liquid scintillation counting (LSC) was done on a Searle Analytic 92 Liquid Scintillation Counter utilizing Aquasol II plus 10% water as the scintillation cocktail. The cpm were converted to dpm using quench curves. Callus tissues which remained after extraction were dried at 105°C for 24 hr. Radiocarbon determinations were made by combustion on a Packard Tri-Carb Sample Oxidizer B306.

Experimental Design

Each of the test compounds ^{14}C aldicarb , ^{14}C aldicarb sulfoxide and ^{14}C aldicarb sulfone were applied individually to cultures of citron callus tissues and potato tuber callus tissues. These can be grouped as two sets of experiments: the three test compounds in citron callus tissue, designated as the citron experiments, and the three compounds in tuber callus tissue, designated as the tuber experiments.

The citron and tuber experiments were designed to obtain results that could be directly compared. Tissue and control samples were maintained under the same environmental conditions using a water jacketed incubator. For the duration of the experiments, the temperature was held at 28°C and the samples were maintained in complete darkness. The conditions of complete darkness reduced or

eliminated degradation of the test compounds by photodecomposition mechanisms. All tissue samples were allowed to stabilize after subculturing for the same time period before treatment. Each sample consisted of three pieces of callus tissue weighing approximately 0.5 to 1.0 g each in an Erlenmeyer flask containing 50 mL of solidified agar medium. Control flasks containing solidified agar media with no tissue were also treated. Each flask received an application rate of approximately 4.0 ppm, based on tissue weight, which represented approximately 300,000 dpm (4 to 5 ug). The ^{14}C material, either aldicarb, the sulfoxide or the sulfone, was prepared using aseptic technique in sterile water and pipetted onto the three tissue pieces in 10 uL aliquots (approximately 100,000 dpm per piece). The control agar received the same treatment, 3 X 100,000 dpm aliquots per flask. At each sampling period three flasks of tissue and three flasks of control agar were analyzed. The sampling periods for the citron callus experiment were: aldicarb, 0.04, 1, 2 and 5 days; sulfoxide and sulfone, 0.04, 1, 2, 5, 8, 13, 20 and 27 days. The sampling intervals for the tuber callus experiment were the same for aldicarb, sulfoxide and sulfone, 0.04, 1, 5, 13 and 27 days. Table 4-2 and 4-3 show the wet weight of citron and tuber callus tissues at the various sampling periods, respectively.

Sample Analysis

In the tissue flasks the callus tissue and agar on which the tissue was grown were analyzed separately. Tissue samples were extracted with ethanol:water (1:1) in a Waring blender. The solvent-tissue homogenates were centrifuged to separate the solid tissue and the supernatant was poured through a suction filter apparatus. The extracted tissue was resuspended in ethanol:water (1:1) and the supernatant filtered two more times. The tissue was suspended in a final aliquot of ethanol:water (1:1) and the suspension was filtered. The centrifuge bottles were then rinsed three times with ethanol:water (1:1), the rinses were filtered and combined with the other extracts.

The agar medium on which the tissue was grown and the control agar were solubilized in ethanol:water (1:1) and poured into separatory funnels. The flasks were rinsed twice with ethanol:water (1:1) and the rinses were also poured into the separatory funnels. The crude tissue extracts and agar extracts were partitioned eight times with chloroform:acetonitrile (3:1). The chloroform fractions were passed through sodium sulfate and combined and concentrated to approximately 1.0 mL using a rotary evaporator. The concentrates were quantitatively transferred to 15 mL centrifuge tubes and concentrated to 200 uL. Aliquots of the final chloroform concentrates were spotted on TLC plates.

Radiocarbon analysis of the extracts was done at various stages of the extraction procedure for all samples. First, a 1.0 mL sample was taken from the crude extract prior to partitioning. Second, a 1.0 mL sample was taken from the total chloroform phase after partitioning. Third, a 1.0 mL sample was taken from the aqueous phase remaining after partitioning. Fourth, a 100 uL sample was taken of the chloroform extract after transfer to the 15 mL centrifuge tube and the volume adjusted to 10 mL.

Identification of Conversion Products

Organic phase concentrates were spotted on TLC plates and developed to 10 cm. Areas of ^{14}C were detected by placing the plates in contact with Kodak SB-5 X-ray film for autoradiography. Each ^{14}C spot was scraped, eluted with Aquasol II plus 10% water scintillation cocktail and counted to determine the percentage distribution of ^{14}C. Radiolabeled standards of aldicarb, aldicarb sulfoxide and aldicarb sulfone were spotted on all sample TLC plates. The identity of the non-labeled reference standards (T, T1, T2, T1O, T2O, T1N and T2N) was confirmed by EI/MS as described above.

The R_f values of the above reference compounds were directly compared to sample R_f values in the 5 TLC solvent systems for identification. Identification of the alcohols, amides and acids was by comparison of sample R_f

values to literature R_f values (Bartley, et al., 1970 and Coppedge et al., 1967).

Results

Physical Characteristics of Callus Tissue

Citron callus tissue. The citron callus tissue was a relatively hard material which required cutting with a scalpel to obtain explant slices. The rate of growth was relatively slow with a typical sample increasing from approximately 1.0 g to 4.0 g over the 27 day sampling period (Table 4.2). The pH of the citron callus tissue was approximately 5.5. The average percentage of water in the callus determined on 9 callus pieces (approximately 0.7 gm of each) was 90.37% (s.d. = 0.94%). Determination of the extracted dry weight of the tissue was done by weighing the dried tissue after extraction. There was approximately 6.46% tissue residue remaining after extraction.

Potato tuber callus tissue. The tuber callus was a friable tissue from which explants were obtained using a spatula. The rate of growth of tuber callus was relatively fast with a typical sample increasing from approximately 2.5 g to approximately 18.0 g over the 27 day sampling period (Table 4-3). The pH of the tuber callus tissue was approximately 5.7. The average percentage of water in the tuber callus was 94.64% (sd = 0.57%) as determined on 9

Table 4-2. Citron Callus Tissue Weight (Grams) at Various Sampling Periods.

Sample Period (days)	Aldicarb	Sulfoxide	Sulfone
0.04	1.240 ± 0.208[a]	0.651 ± 0.293	1.349 ± 0.212
1	1.193 ± 0.186	1.135 ± 0.169	1.818 ± 0.301
2	1.430 ± 0.180	2.385 ± 0.359	1.391 ± 0.126
5	1.536 ± 0.253	2.104 ± 0.053	2.021 ± 0.029
8	-	2.698 ± 0.896	2.536 ± 0.440
13	-	2.911 ± 0.533	3.010 ± 0.208
20	-	4.292 ± 0.159	4.208 ± 0.524
27	-	3.625 ± 0.071	4.776 ± 0.528

[a] Mean of triplicate samples and standard deviation.

Table 4-3. Tuber Callus Tissue Weight (Grams) at Various Sampling Periods.

Sample Period (days)	Aldicarb	Sulfoxide	Sulfone
0.04	3.486 ± 0.588	1.896 ± 0.564	2.773 ± 0.503
1	2.662 ± 0.259	2.001 ± 0.177	2.166 ± 0.245
5	-	2.501 ± 0.517	2.687 ± 0.373
13	7.160 ± 2.974	5.890 ± 1.339	5.878 ± 2.992
27	20.816 ± 4.895	17.886 ± 8.916	18.399 ± 8.922

[a] Mean of triplicate samples and standard deviation.

callus pieces approximately 1.0 g each. There was approximately 0.72% tissue residue remaining after extraction.

Total Radiocarbon Distribution

Total radiocarbon recovery from citron callus tissue and tuber callus tissue experiments. The total percentage of recovered ^{14}C in citron and tuber callus tissue flasks is presented in Tables 4-4 and 4-5, respectively. In the citron experiment the percentage ^{14}C recovered generally decreased as time post application increased for all three compounds. Higher total recoveries were observed in the sulfoxide and sulfone experiments than in the aldicarb experiment. In the tuber callus experiment there was less of a decrease in the total recovered ^{14}C than in the citron experiment. The highest recoveries were from the sulfone application, followed by sulfoxide application and then the aldicarb application.

The total percentage recoveries in the control flasks are shown in Tables 4-6 and 4-7, respectively. For both experiments the control recoveries ranged between 82% and 100% for all three compounds.

Distribution of recovered radiocarbon between tissue and agar. The percentage distributions of recovered ^{14}C in the tissue and agar for the citron and tuber callus experiments are presented in Tables 4-8, 4-9, 4-10 and 4-11. The distributions were determined at all sampling periods by analysis of the crude tissue and agar extracts

Table 4-4. Percentage of Radiocarbon Recovered (of the Applied) from Citron Callus Tissue Flasks Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone.

Sample Period (days)	Total Percentage Recovered Citron Callus		
	Aldicarb	Sulfoxide	Sulfone
0.04	90.9 ± 4.4[a]	100.0 ± 2.0	95.2 ± 1.3
1	91.0 ± 4.4	98.7 ± 1.5	94.2 ± 4.8
2	84.3 ± 7.5	94.3 ± 1.7	92.3 ± 1.3
5	80.8 ± 5.3	98.7 ± 2.9	87.1 ± 1.5
8	-	91.2 ± 4.4	85.6 ± 2.4
13	-	88.7 ± 1.5	84.6 ± 4.4
20	-	91.3 ± 2.6	85.4 ± 1.1
27	-	87.0 ± 2.0	81.1 ± 2.9

[a] Mean of triplicate samples and standard deviation.

Table 4-5. Percentage of Radiocarbon Recovered (of the Applied) from Tuber Callus Tissue F l a s k s Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone.

Sample Period (days)	Percentage Recovered Tuber Callus		
	Aldicarb	Sulfoxide	Sulfone
0.04	91.9 ± 1.4[a]	95.9 ± 4.5	102.1 ± 3.4
1	84.5 ± 4.2	95.2 ± 1.9	97.6 ± 1.6
5	73.3 ± 8.8	91.9 ± 1.4	84.0 ± 3.7
13	90.2 ± 4.9	88.8 ± 6.6	100.6 ± 5.7
27	85.1 ± 13.5	94.6 ± 4.2	99.1 ± 5.3

[a] Mean of triplicate samples and standard deviation.

Table 4-6. Percentage of Radiocarbon Recovered (of the Applied) from Control Flasks Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone in the Citron Callus Experiment.

Sample Period (days)	Percentage Recovered Control		
	Aldicarb	Sulfoxide	Sulfone
0.04	95.6 ± 1.7[a]	90.1 ± 2.2	93.4 ± 4.5
1	97.2 ± 3.3	93.1 ± 2.1	100.6 ± 0.7
2	95.7 ± 3.0	96.8 ± 4.8	85.7 ± 5.2
5	93.9 ± 0.6	96.4 ± 5.4	88.0 ± 2.7
8	-	92.8 ± 2.0	86.8 ± 4.3
13	-	100.4 ± 3.8	87.9 ± 3.5
20	-	91.1 ± 2.4	87.0 ± 1.6
27	-	92.6 ± 2.3	95.6 ± 3.8

[a] Mean of triplicate samples and standard deviation.

Table 4-7. Percentage of Radiocarbon Recovered (of the Applied) from Control Flasks Treated with Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone in the Tuber Callus Experiment.

Sample Period (days)	Percent Recovered		
	Aldicarb	Sulfoxide	Sulfone
0.04	85.6 ± 1.5	89.9 ± 3.3	100.4 ± 0.9
1	84.3 ± 3.7	90.1 ± 1.7	93.6 ± 1.8
5	88.3 ± 6.6	93.5 ± 5.4	87.9 ± 1.7
13	78.9 ± 7.7	89.1 ± 3.4	93.1 ± 0.7
27	81.6 ± 6.0	92.5 ± 1.9	96.6 ± 1.6

[a] Mean of triplicate samples and standard deviation.

Table 4-8. Percentage of Radiocarbon Recovered in the Citron Callus Tissue when Treated with Aldicarb, Aldicarb Sulfoxide or Aldicarb Sulfone.

Sample Period (days)	Percentage Recovered Citron Tissue		
	Aldicarb	Sulfoxide	Sulfone
0.04	80.3 ± 1.0[a]	83.1 ± 14.2	85.4 ± 11.8
1	34.8 ± 5.8	91.9 ± 6.5	55.7 ± 25.5
2	38.4 ± 23.0	68.6 ± 6.1	46.7 ± 17.3
5	7.4 ± 1.4	35.2 ± 11.3	36.1 ± 8.2
8	-	47.9 ± 11.6	21.8 ± 4.5
13	-	61.8 ± 6.8	19.9 ± 2.4
20	-	32.3 ± 14.8	21.7 ± 5.1
27	-	20.0 ± 5.9	32.2 ± 9.7

a Mean of triplicate samples and standard deviation.

Table 4-9. Percentage of Radiocarbon Recovered in the Agar on which Citron Callus Tissues were Grown in Flasks Treated with Aldicarb, Aldicarb Sulfoxide or Aldicarb Sulfone.

Sample Period (days)	Percentage Recovered Citron Agar		
	Aldicarb	Sulfoxide	Sulfone
0.04	19.8 ± 1.0[a]	16.9 ± 14.2	14.6 ± 11.8
1	65.2 ± 5.8	7.8 ± 6.5	44.3 ± 25.5
2	61.6 ± 23.0	31.1 ± 6.0	53.3 ± 17.3
5	92.6 ± 1.4	64.7 ± 11.3	63.7 ± 8.4
8	-	52.2 ± 11.6	78.2 ± 4.5
13	-	38.2 ± 6.8	80.1 ± 2.4
20	-	64.7 ± 14.8	78.3 ± 5.1
27	-	80.0 ± 5.9	69.7 ± 12.0

[a] Mean of triplicate samples and standard deviation.

Table 4-10. Percentage of Radiocarbon Recovered in the Tuber Callus Tissue when Treated with Aldicarb, Aldicarb Sulfoxide or Aldicarb Sulfone.

Sample Period (days)	Percentage Recovered in Tissue		
	Aldicarb	Sulfoxide	Sulfone
0.04	96.5 ± 2.4[a]	96.6 ± 1.0	98.8 ± 0.6
1	54.3 ± 1.8	50.2 ± 6.1	45.1 ± 10.6
5	25.8 ± 1.1	14.9 ± 4.2	8.7 ± 2.0
13	53.3 ± 9.1	20.4 ± 4.8	18.8 ± 9.1
27	78.7 ± 7.8	42.4 ± 9.4	54.3 ± 20.3

[a] Mean of triplicate samples and standard deviation.

Table 4-11. Percentage of Radiocarbon Recovered in the Agar on which Tuber Callus Tissue was Grown in Flasks Treated with Aldicarb, Aldicarb Sulfoxide or Aldicarb Sulfone.

Sample Period (days)	Percentage Recovered in Agar		
	Aldicarb	Sulfoxide	Sulfone
0.04	3.5 ± 2.4[a]	3.4 ± 1.0	1.3 ± 0.6
1	45.7 ± 1.8	49.3 ± 6.1	54.9 ± 10.6
5	74.2 ± 1.1	85.1 ± 4.2	91.3 ± 2.0
13	46.7 ± 9.1	79.6 ± 4.8	81.2 ± 9.1
27	21.3 ± 7.8	57.6 ± 9.4	45.7 ± 20.3

[a] Mean of triplicate samples and standard deviation.

for ^{14}C content. In the citron experiment there was a decrease in the ^{14}C in the tissue as time post application increased for all three compounds (Table 4-8). Tissue ^{14}C for the aldicarb application decreased from 80.3% at 0.04 days to 7.4% at 5 days. In both the sulfoxide and sulfone citron experiments, the percentages of ^{14}C in the tissue decreased from approximately 84% at 0.04 days to a range of 20.0% to 32.2% at 27 days. The agar ^{14}C increased as time post application increased (Table 4-9). Therefore, there was a general movement of ^{14}C from the tissue to the agar.

In the tuber callus experiment the percentage of ^{14}C in the tissue decreased from approximately 97% at 0.04 days to approximately 14% at 5 days and then increased until the 27 day sample (42.4% to 78.7%) post application (Table 4-10). This ^{14}C movement trend was observed for all three compounds in the tuber callus experiments. Consequently, there was an initial increase in agar ^{14}C during the early sampling periods followed by a decrease at the later sampling periods (Table 4-11).

Metabolic Radiocarbon Distribution

The tissue and the agar were analyzed individually for the ^{14}C metabolic products. The agar from the control flasks was also analyzed for conversion products. Aqueous soluble ^{14}C materials were not analyzed further. Four sets of data are presented to show the percentage distribution of metabolites for each experiment. The four sets of data

represent the metabolic distribution of: ^{14}C in the whole treated tissue flask (tissue ^{14}C plus agar ^{14}C), ^{14}C in the callus tissue, ^{14}C in the agar on which the callus tissue was grown, and ^{14}C in the control flask agar. Table 4-12 indicates the half lives of parent compound for citron and tuber callus experiments, respectively.

Aldicarb experiment in citron and tuber callus. The percentage distribution of metabolites, aqueous soluble radiocarbon and unextractable residue in the whole tissue flask is presented in Table 4-13 for the aldicarb citron experiment. Parent aldicarb was present at all sampling periods but the percentage rapidly declined from 75% to 21% between 0.04 days and 1 day. By 5 days post application 15% of the ^{14}C in the flask was parent aldicarb. The major metabolite was the sulfoxide which was formed rapidly. By 1 day the percentage of sulfoxide had reached 68% and remained at that level through 5 days post application. Small percentages of sulfone (2.4%), sulfoxide oxime (1.2%), sulfoxide and sulfone nitrile (1.2% and 0.6%, respectively) and sulfoxide alcohol (0.5%) were also detected at 5 days. Aqueous soluble ^{14}C increased from 1.5% at 0.04 days to 10.8% at 5 days and bound residues remained below 0.6%.

Data for the citron tissue and agar are presented in Tables 4-14 and 4-15, respectively which show no major differences in the metabolic profiles for these two

Table 4-12. Pseudo First Order Rate Constants, k, Half Lives and Coefficients of Determination for Aldicarb, Aldicarb Sulfoxide and Aldicarb Sulfone in Citron and Potato Tuber Callus Tissues.

	Aldicarb		Sulfoxide		Sulfone	
	A & T[a]	T[b]	A & T	T	A & T	T
Citron						
k[c]	0.247[e]	0.470	0.010	0.032	0.011	0.032
$t_{\frac{1}{2}}$[d]	2.8	1.5	67.9	21.6	60.6	21.8
r^2	0.530	0.584	0.706	0.824	0.958	0.962
Tuber						
k	0.100	0.151	0.025	0.056	0.064	0.117
$t_{\frac{1}{2}}$[d]	6.9	4.6	27.5	12.4	10.9	5.9
r^2	0.977	0.905	0.994	0.984	0.895	0.988

[a] Agar and tissue radiocarbon combined.

[b] Tissue radiocarbon.

[c] Pseudo first order rate constant, k, days $^{-1}$.

[d] Half-life, days.

[e] See text for explanation.

Table 4-13. Percentage Distribution of Radiocarbon in Citron Callus Tissues and Agar (Combined) Treated with Aldicarb.

	Aldicarb Citron Tissue and Agar			
Compound	0.04 [a]	1	2	5
T	74.7 ± 4.2[b]	20.9 ± 3.4	17.3 ± 6.5	15.4 ± 3.0
T1	21.9 ± 3.8	68.3 ± 3.5	67.2 ± 6.0	67.3 ± 4.5
T2	0.3 ± 0.1	1.3 ± 0.1	1.7 ± 1.2	2.4 ± 0.8
T1 0	0.2 ± 0.04	0.5 ± 0.1	0.8 ± 0.3	1.2 ± 0.3
T1 AM	-[c]	-	0.1 ± 0.1	-
T2 AM	-	-	0.1 ± 0.04	-
T1 N	0.6 ± 0.1	1.2 ± 0.1	2.2 ± 2.2	1.2 ± 0.4
T2 N	0.6 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.2
T1 ALC	-	0.4 ± 0.1	0.4 ± 0.3	0.5 ± 0.2
Unknown 1	-	-	0.1 ± 0.1	-
Polar	0.1 ± 0.1	0.3 ± 0.03	0.1 ± 0.04	0.2 ± 0.2
Aqueous	1.5 ± 0.2	6.0 ± 0.3	8.9 ± 1.8	10.8 ± 1.7
Bound	0.1 ± 0.01	0.3 ± 0.02	0.6 ± 0.2	0.4 ± 0.1

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-14. Percentage Distribution of Radiocarbon in Citron Callus Tissues Treated with Aldicarb.

	Aldicarb Citron Tissue			
Compound	0.04 [a]	1	2	5
T	71.8 ± 4.9[b]	6.6 ± 1.6	6.8 ± 3.8	3.7 ± 1.0
T1	24.1 ± 4.6	80.0 ± 1.7	71.7 ± 5.1	71.2 ± 2.0
T2	0.3 ± 0.1	2.0 ± 0.3	2.5 ± 1.9	4.3 ± 0.2
T1 0	0.3 ± 0.1	0.7 ± 0.2	0.8 ± 0.4	1.6 ± 0.4
T1 AM	-[c]	-	0.1 ± 0.2	-
T2 AM	-	-	0.2 ± 0.02	-
T1 N	0.7 ± 0.1	1.4 ± 0.1	2.6 ± 2.9	-
T2 N	0.6 ± 0.0	1.2 ± 0.6	0.5 ± 0.1	1.3 ± 0.4
T1 ALC	-	0.5 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
Unknown 1	-	-	0.2 ± 0.1	-
Polar	-	-	-	-
Aqueous	1.3 ± 0.2	6.6 ± 0.1	12.4 ± 1.6	12.6 ± 1.6
Bound	0.1 ± 0.01	1.0 ± 0.2	1.7 ± 0.5	4.9 ± 0.5

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-15. Percentage Distribution of Radiocarbon in Agar on which Citron Callus Tissues were Grown and Treated with Aldicarb.

	Aldicarb Citron Agar			
Compound	0.04 [a]	1	2	5
T	85.7 ± 1.1[b]	28.4 ± 5.2	24.7 ± 8.3	12.3 ± 4.5
T1	13.0 ± 0.9	62.2 ± 4.9	64.8 ± 7.8	71.2 ± 2.8
T2	-[c]	1.0 ± 0.0	1.3 ± 1.0	2.1 ± 0.5
T1 0	-	0.4 ± 0.3	0.7 ± 0.2	1.0 ± 0.1
T1 AM	-	-	-	-
T2 AM	-	-	-	-
T1 N	0.2 ± 0.3	1.0 ± 0.1	1.3 ± 0.9	0.9 ± 0.1
T2 N	0.2 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.04
T1 ALC	-	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
Unknown 1	-	-	-	-
Polar	0.04 ± 0.05	0.3 ± 0.1	0.3 ± 0.03	0.2 ± 0.2
Aqueous	0.8 ± 0.03	5.8 ± 0.5	6.1 ± 0.3	11.4 ± 1.9

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

components. The percentage of parent aldicarb was lower in the tissue than in the agar at all sampling periods. Percentages of metabolites were slightly higher in the citron tissue. However, the actual radiocarbon content of the tissue was lower than that in the agar (7.4% in tissue and 92.6% in agar) at 5 days.

The percentage distribution for ^{14}C conversion products in citron control agar are presented in Table 4-16. Parent aldicarb was relatively stable when exposed to only the agar. The percentage of aldicarb in the control flask was 94% to 95% throughout the 5 days. There was approximately 3% sulfoxide present at all sampling periods. All other conversion products, including aqueous ^{14}C, were below 1.0%.

Data from aldicarb application to citron callus tissue are summarized in Figure 4-1. The percentage of parent aldicarb in the control agar, the percentage of parent aldicarb in the whole tissue flask (tissue ^{14}C plus agar ^{14}C) and the percentage of the major metabolic product, sulfoxide, in the whole tissue flask are shown. This illustrates the rapid conversion of aldicarb to the sulfoxide. This rapid decrease in parent does not follow first order decay so a true half-life could not be calculated. The low coefficients of determination (Table 4-12) indicated that the data did not fit first order kinetics. From Figure 4-1 it was evident that over half of the parent

Table 4-16. Percentage Distribution of Radiocarbon in Citron Control Agar Treated with Aldicarb.

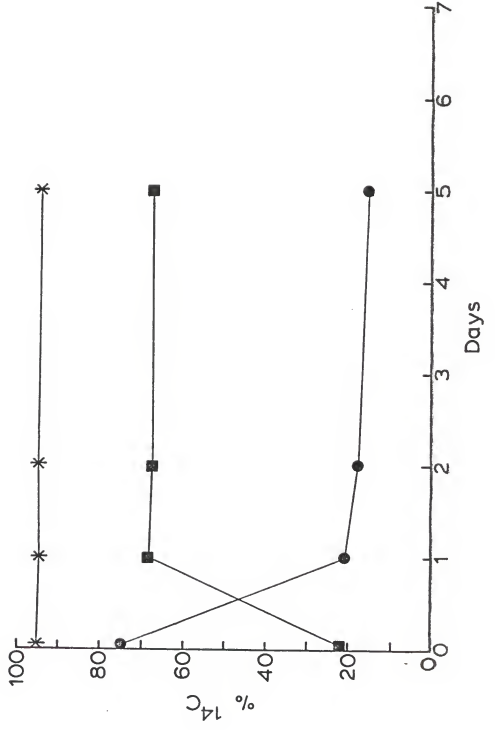
	Aldicarb Citron Control			
Compound	0.04 [a]	1	2	5
T	95.3 ± 0.2[b]	94.4 ± 0.7	94.8 ± 0.1	94.2 ± 0.1
T1	2.8 ± 0.3	3.2 ± 0.5	3.2 ± 0.3	3.4 ± 0.2
T2	0.2 ± 0.1	0.4 ± 0.03	0.5 ± 0.04	0.5 ± 0.1
T1 0	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
T1 AM	-[c]	-	-	-
T2 AM	0.2 ± 0.3	-	-	-
T1 N	0.6 ± 0.03	0.5 ± 0.1	0.6 ± 0.01	0.6 ± 0.1
T2 N	-	-	-	-
T1 ALC	-	0.3 ± 0.2	0.1 ± 0.0	-
Unknown 1	-	-	-	-
Polar	0.1 ± 0.04	0.2 ± 0.1	0.01 ± 0.01	0.2 ± 0.2
Aqueous	0.3 ± 0.3	0.4 ± 0.02	0.3 ± 0.1	0.6 ± 0.1

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Figure 4-1. Percentage of parent aldicarb and major metabolites in citron callus and control. Aldicarb in control (*), aldicarb in tissue flask (●), sulfoxide in tissue flask (■).


100
80
60
40
20
0
% 14C
0
1
2
3
4
5
6
7
Days

aldicarb was degraded in less than one day. The percentage of parent aldicarb in the control remained constant.

The percentage distribution of metabolites, aqueous soluble ^{14}C and unextractable residue in the whole tissue flask is shown in Table 4-17 for the tuber callus aldicarb experiment. Parent aldicarb was present at all sampling periods and decreased from 93.68% at 0.04 days to 5.68% at 27 days. The sulfoxide was the major metabolite but only reached a maximum value of 15% at 5 and 13 days post application. The percentage of sulfoxide decreased during the 13 to 27 days interval to 9.7%. Sulfoxide oxime and sulfoxide nitrile were the other major metabolites detected at 27 days, representing 8.0% and 2.5% of the ^{14}C, respectively. Aqueous soluble ^{14}C increased from 1.3% at 0.04 days to 70.5% at 27 days. Bound residues did not exceed 1.1% at any of the sampling periods.

The percentage distributions of metabolites in the tissue and agar independently are presented in Tables 4-18 and 4-19, respectively. The metabolites found in the tuber callus tissue and agar on which it was grown were similar. However, aldicarb represented a much smaller percentage of the tissue radiocarbon (1.4%) than of the agar radiocarbon (17.5%). The percentages of other metabolites were higher in the agar than in the tissue whereas in the citron callus aldicarb experiment, metabolite percentages were higher in the tissue. At 27 days the sulfoxide percentage in the

Table 4-17. Percentage Distribution of Radiocarbon in Tuber Callus Tissues and Agar (Combined) Treated with Aldicarb.

	Aldicarb Tuber Tissue and Agar				
Compound	0.04 [a]	1	5	13	27
T	93.5 ± 0.6[b]	74.3 ± 1.5	57.8 ± 4.6	35.3 ± 8.6	5.6 ± 4.3
T1	2.7 ± 0.4	12.5 ± 1.2	15.4 ± 3.8	14.6 ± 2.6	9.7 ± 2.8
T2	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 0.3	-
T1 O	0.5 ± 0.2	1.3 ± 0.2	1.0 ± 0.5	2.7 ± 1.7	8.0 ± 2.9
T1 AM	0.3 ± 0.3	0.03 ± 0.1	0.04 ± 0.03	0.2 ± 0.2	0.1 ± 0.1
T2 AM	-[c]	0.3 ± 0.1	0.4 ± 0.2	-	-
T1 N	0.4 ± 0.1	0.4 ± 0.2	1.9 ± 0.3	2.1 ± 0.7	2.5 ± 0.7
T2 N	-	0.3 ± 0.1	0.1 ± 0.04	-	-
T1 ALC	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.9 ± 0.2
Unknown 1	-	0.02 ± 0.03	0.04 ± 0.04	0.1 ± 0.01	-
Polar	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.2	1.6 ± 1.5
Aqueous	1.3 ± 0.2	7.0 ± 1.1	22.0 ± 0.6	42.5 ± 4.1	70.5 ± 5.8
Bound	0.1 ± 0.03	0.1 ± 0.03	0.4 ± 0.01	0.7 ± 0.1	1.1 ± 0.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-18. Percentage Distribution of Radiocarbon in Tuber Callus Tissues Treated with Aldicarb.

	Aldicarb Tuber Tissue				
Compound	0.04 [a]	1	5	13	27
T	94.0 ± 0.8[b]	68.6 ± 1.6	13.3 ± 3.2	5.2 ± 1.4	1.4 ± 1.2
T1	2.4 ± 0.4	14.6 ± 1.5	4.9 ± 4.2	8.3 ± 2.8	3.6 ± 2.2
T2	0.4 ± 0.1	0.4 ± 0.1	-	0.7 ± 0.2	-
T1 0	0.5 ± 0.2	1.7 ± 0.4	3.2 ± 1.6	4.3 ± 2.2	5.9 ± 2.2
T1 AM	0.3 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	-	0.03 ± 0.1
T2 AM	-[c]	0.4 ± 0.02	0.1 ± 0.1	-	-
T1 N	0.4 ± 0.1	0.4 ± 0.1	1.9 ± 0.3	2.8 ± 1.3	2.0 ± 1.1
T2 N	-	0.6 ± 0.2	-	-	-
T1 ALC	0.3 ± 0.1	0.4 ± 0.3	0.1 ± 0.03	0.6 ± 0.1	0.7 ± 0.6
Unknown 1	-	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	-
Polar	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.5 ± 0.2	1.9 ± 1.9
Aqueous	1.3 ± 0.2	12.2 ± 1.8	74.3 ± 3.2	76.0 ± 5.5	83.1 ± 1.2
Bound	0.1 ± 0.03	0.1 ± 0.1	1.5 ± 0.03	1.4 ± 0.2	1.4 ± 0.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-19. Percentage Distribution of Radiocarbon in Agar on which Tuber Callus Tissues were Grown and Treated with Aldicarb.

	Aldicarb Tuber Agar				
Compound	0.04 [a]	1	5	13	27
T	74.3 ± 7.8[b]	87.1 ± 0.8	73.1 ± 4.6	50.3 ± 9.8	17.5 ± 10.9
T1	11.5 ± 1.4	10.1 ± 0.7	19.1 ± 3.8	27.3 ± 5.0	20.6 ± 5.4
T2	-[c]	0.1 ± 0.1	0.3 ± 0.1	1.4 ± 0.3	-
T1 0	-	0.9 ± 0.2	0.2 ± 0.1	3.8 ± 1.9	12.3 ± 5.8
T1 AM	-	-	-	0.4 ± 0.4	0.3 ± 0.2
T2 AM	-	0.3 ± 0.2	0.6 ± 0.3	-	-
T1 N	-	0.4 ± 0.3	1.9 ± 0.3	3.1 ± 1.1	6.5 ± 2.4
T2 N	-	-	0.2 ± 0.1	-	-
T1 ALC	-	-	0.4 ± 0.2	0.8 ± 0.2	2.9 ± 0.2
Unknown 1	-	-	-	0.1 ± 0.1	-
Polar	8.7 ± 4.1	0.4 ± 0.1	0.6 ± 0.4	0.4 ± 0.2	0.5 ± 0.3
Aqueous	5.5 ± 2.5	0.7 ± 0.2	3.6 ± 0.4	12.4 ± 2.4	39.4 ± 7.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

agar was 20.6% compared to 3.6% in the tissue. Aqueous soluble ^{14}C represented 83.1% of the tissue radiocarbon and 39.4% of the agar radiocarbon.

The percentage distribution of metabolites in the aldicarb tuber control agar is shown in Table 4-20. The percentage of aldicarb remained relatively constant, ranging from 94.1% to 97.2% throughout the sampling periods. Sulfoxide represented 1.5% to 3.0% of the radiocarbon. Small amounts (less than 0.5% each) of other conversion products were also detected. Aqueous soluble ^{14}C increased from 0.6% to 2.6% over the sampling intervals.

Data which summarize the major metabolic activity in tuber callus tissues treated with aldicarb are illustrated in Figure 4-2. The data are percentages of the ^{14}C material in the whole flask. Aldicarb rapidly degraded in the tuber callus with a half life of 6.9 days (Table 4-12) while the parent was relatively stable in control agar.

Aldicarb sulfoxide experiment in citron and tuber callus. The percentage distribution of metabolites, aqueous soluble ^{14}C and unextractable residue in the whole tissue flask are shown in Table 4-21 for the citron callus sulfoxide experiment. Parent sulfoxide was the major ^{14}C material at all sampling periods. The sulfoxide percentage decreased from 93.9% at 0.04 days to 67.1% at 27 days post application. The major metabolites at 27 days were

Table 4-20. Percentage Distribution of Radiocarbon in Tuber Callus Control Agar Treated with Aldicarb.

	Aldicarb Tuber Control				
Compound	0.04 [a]	1	5	13	27
T	95.0 ± 0.3[b]	97.2 ± 0.1	96.0 ± 0.2	95.1 ± 0.2	94.0 ± 0.5
T1	3.1 ± 0.4	1.6 ± 0.04	1.8 ± 0.2	1.6 ± 0.1	1.9 ± 0.2
T2	-[c]	-	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
T1 0	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.03	0.5 ± 0.1	0.3 ± 0.01
T1 AM	-	-	-	-	-
T2 AM	-	-	-	-	-
T1 N	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.02	0.3 ± 0.1	0.4 ± 0.1
T2 N	-	-	0.1 ± 0.2	-	-
T1 ALC	-	-	0.04 ± 0.1	-	0.2 ± 0.1
Unknown 1	-	-	-	-	-
Polar	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.4
Aqueous	0.6 ± 0.1	0.1 ± 0.03	1.0 ± 0.1	1.9 ± 0.3	2.6 ± 0.6

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Figure 4-2. Percentage of parent aldicarb and major metabolites in potato tuber callus and control. Aldicarb in control (*), aldicarb in tissue flask (●), sulfoxide in tissue flask (■) and aqueous soluble radiocarbon in tissue flask (▲).

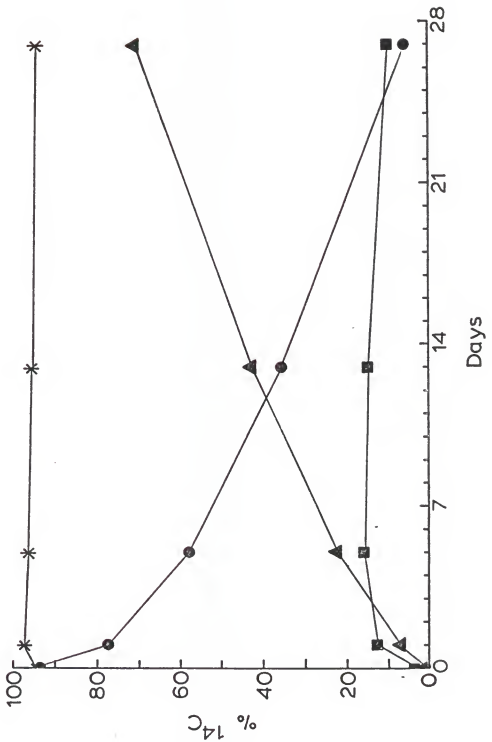

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Table 4-21. Percentage Distribution of Radiocarbon in Citron Callus Tissues and Agar (Combined) Treated with Aldicarb Sulfoxide.

	Sulfoxide Citron Tissue and Agar			
Compound	0.04 [a]	1	2	5
T1	93.9 ± 0.3[b]	90.2 ± 2.0	87.7 ± 0.5	83.9 ± 1.5
T2	0.5 ± 0.4	0.6 ± 0.4	0.6 ± 0.1	0.9 ± 0.1
T1 0	0.2 ± 0.1	0.6 ± 0.3	0.4 ± 0.04	1.2 ± 0.1
T1 AM	-[c]	0.9 ± 0.3	0.4 ± 0.1	1.0 ± 0.2
T2 AM	0.04 ± 0.03	0.7 ± 0.4	0.7 ± 0.1	-
T1 N	0.01 ± 0.02	1.0 ± 0.4	1.4 ± 0.5	2.9 ± 1.1
T2 N	-	-	-	-
T1 ALC	-	-	0.1 ± 0.1	0.1 ± 0.03
Unknown 1	-	-	-	-
Polar	0.4 ± 0.4	0.8 ± 0.3	0.2 ± 0.1	0.5 ± 0.1
Aqueous	4.9 ± 0.1	4.8 ± 0.4	8.0 ± 0.2	9.2 ± 0.5
Bound	0.1 ± 0.01	0.3 ± 0.02	0.5 ± 0.1	0.3 ± 0.1

Table 4-21 continued.

	Sulfoxide Citron Tissue and Agar			
Compound	8[a]	13	20	27
T1	80.0 ± 2.9[b]	69.8 ± 0.7	81.2 ± 3.4	66.9 ± 10.4
T2	2.1 ± 1.5	3.0 ± 0.6	2.7 ± 0.9	3.2 ± 1.4
T1 0	3.1 ± 0.9	4.0 ± 0.9	0.5 ± 0.4	3.0 ± 0.9
T1 AM	1.2 ± 0.8	1.4 ± 0.5	1.0 ± 0.2	4.8 ± 1.2
T2 AM	-[c]	-	-	0.5 ± 0.4
T1 N	4.3 ± 2.2	4.8 ± 1.2	0.2 ± 0.1	6.7 ± 3.0
T2 N	-	-	-	0.3 ± 0.3
T1 ALC	-	-	0.01 ± 0.01	0.9 ± 0.7
Unknown 1	-	0.8 ± 0.1	0.8 ± 0.4	0.4 ± 0.5
Polar	0.2 ± 0.2	0.4 ± 0.1	0.5 ± 0.4	1.8 ± 1.6
Aqueous	8.9 ± 0.5	15.7 ± 0.8	12.7 ± 1.8	11.3 ± 3.4
Bound	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.02

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

sulfoxide nitrile (6.7%), sulfoxide amide (4.8%) and sulfoxide oxime (3.2%). Conversion of the sulfoxide to sulfone was not a major pathway in citron callus since the percentage of sulfone was 3.2% at 27 days. Aqueous soluble radiocarbon increased from 4.9% to 11.3% by 27 days. Bound residues were less than 0.5% of the total ^{14}C at all sampling periods.

The percentage distributions of metabolites for the citron callus tissue radiocarbon and the agar radiocarbon are presented in Tables 4-22 and 4-23, respectively. By 27 days the percentage of parent sulfoxide was 32.1% of the tissue ^{14}C and 74.6% of the agar ^{14}C. The percentages of major metabolites were higher in the citron tissue than in the agar, as was observed with aldicarb in the citron callus tissue. At 27 days, comparisons of major metabolites in tissue versus agar are sulfoxide amide, 16.1% to 3.1%, sulfoxide nitrile, 8.7% to 6.4% and sulfone, 7.3% to 2.1%.

The distribution of radiocarbon in the control flask from the citron callus sulfoxide experiment is shown in Table 4-24. Parent sulfoxide ranged between 84.0% to 94.7% throughout the sampling period. Major conversion products at 27 days were the sulfoxide nitrile (3.5%) and sulfoxide oxime (2.7%). The percentage of aqueous soluble ^{14}C was relatively constant from 0.04 days to 27 days and ranged between 3.7% and 7.6%.

Table 4-22. Percentage Distribution of Radiocarbon in Citron Callus Tissues Treated with Aldicarb Sulfoxide.

	Sulfoxide Citron Tissue			
Compound	0.04[a]	1	2	5
T1	94.1 ± 0.5[b]	90.2 ± 2.0	86.5 ± 0.6	80.5 ± 0.1
T2	0.7 ± 0.6	0.7 ± 0.5	0.6 ± 0.1	1.2 ± 0.4
T1 0	0.3 ± 0.02	0.7 ± 0.4	0.3 ± 0.04	1.5 ± 0.4
T1 AM	-[c]	1.0 ± 0.4	0.6 ± 0.1	1.6 ± 0.2
T2 AM	0.1 ± 0.1	0.8 ± 0.5	0.7 ± 0.04	-
T1 N	-	1.1 ± 0.4	1.2 ± 0.5	2.2 ± 0.8
T2 N	-	-	-	-
T1 ALC	-	-	0.1 ± 0.1	0.4 ± 0.1
Unknown 1	-	-	-	-
Polar	0.04 ± 0.02	0.7 ± 0.2	0.1 ± 0.1	0.3 ± 0.3
Aqueous	4.7 ± 0.03	4.7 ± 0.6	9.6 ± 0.3	11.8 ± 0.6
Bound	0.1 ± 0.01	0.1 ± 0.02	0.3 ± 0.1	0.5 ± 0.1

Table 4-22 continued.

Compound	Sulfoxide Citron Tissue			
	8	13	20	27
T1	78.2 ± 3.3[b]	68.7 ± 0.6	67.5 ± 1.0	32.0 ± 17.8
T2	3.1 ± 2.1	2.3 ± 0.4	5.4 ± 0.7	7.3 ± 5.3
T1 0	2.1 ± 1.9	4.3 ± 1.5	-	2.0 ± 0.7
T1 AM	1.6 ± 0.3	2.4 ± 1.2	2.9 ± 0.7	16.1 ± 14.8
T2 AM	-[c]	-	-	2.0 ± 1.4
T1 N	3.9 ± 2.5	3.3 ± 0.7	-	8.7 ± 8.2
T2 N	-	-	-	-
T1 ALC	-	-	0.02 ± 0.02	6.2 ± 6.0
Unknown 1	-	0.8 ± 0.2	1.7 ± 0.5	0.4 ± 0.2
Polar	-	0.4 ± 0.1	1.0 ± 0.4	2.0 ± 1.5
Aqueous	10.7 ± 1.5	17.6 ± 1.3	20.4 ± 0.5	22.3 ± 6.5
Bound	0.4 ± 0.2	0.2 ± 0.1	1.1 ± 0.4	1.0 ± 0.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-23. Percentage Distribution of Radiocarbon in Agar on which Citron Callus Tissues were Grown and Treated with Aldicarb Sulfoxide.

	Sulfoxide Citron Agar			
Compound	0.04 [a]	1	2	5
T1	89.1 ± 5.8[b]	86.2 ± 6.2	90.6 ± 2.1	85.7 ± 1.4
T2	-[c]	0.4 ± 0.5	0.7 ± 0.2	0.8 ± 0.1
T1 0	-	0.1 ± 0.1	0.6 ± 0.3	1.2 ± 0.1
T1 AM	-	-	-	0.7 ± 0.2
T2 AM	-	-	0.8 ± 0.3	-
T1 N	0.2 ± 0.3	0.8 ± 1.0	2.1 ± 1.4	3.5 ± 1.4
T2 N	-	-	-	-
T1 ALC	-	-	-	-
Unknown 1	-	-	-	-
Polar	5.2 ± 6.2	7.3 ± 7.3	0.5 ± 0.2	0.5 ± 0.1
Aqueous	5.5 ± 0.5	5.2 ± 0.8	4.7 ± 0.3	7.6 ± 0.4

Table 4-23 continued.

Compound	Sulfoxide Citron Agar			
	8[a]	13	20	27
T1	82.0 ± 2.0[b]	71.3 ± 1.9	89.2 ± 0.7	74.6 ± 9.0
T2	0.8 ± 0.6	3.8 ± 1.6	0.9 ± 0.2	2.1 ± 0.5
T1 0	3.9 ± 0.6	3.7 ± 0.5	0.7 ± 0.5	3.2 ± 1.1
T1 AM	0.9 ± 1.3	-	0.02 ± 0.03	3.1 ± 0.9
T2 AM	-[c]	-	-	-
T1 N	4.8 ± 1.9	7.6 ± 2.5	0.3 ± 0.2	6.4 ± 3.0
T2 N	-	-	-	0.4 ± 0.4
T1 ALC	-	-	-	-
Unknown 1	-	0.8 ± 0.3	0.2 ± 0.1	0.4 ± 0.6
Polar	0.4 ± 0.4	0.4 ± 0.2	0.1 ± 0.04	1.7 ± 1.7
Aqueous	7.1 ± 1.2	12.4 ± 0.6	8.7 ± 0.1	8.2 ± 2.9

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-24. Percentage Distribution of Radiocarbon in Citron Control Agar Treated With Aldicarb Sulfoxide.

	Sulfoxide Citron Control			
Compound	0.04[a]	1	2	5
T1	92.4 ± 0.7[b]	94.8 ± 0.2	93.3 ± 0.7	91.6 ± 1.0
T2	-[c]	-	0.2 ± 0.1	0.3 ± 0.03
T1 0	0.5 ± 0.1	-	0.5 ± 0.1	0.6 ± 0.1
T1 AM	-	-	-	0.3 ± 0.2
T2 AM	-	-	0.2 ± 0.04	-
T1 N	-	-	0.2 ± 0.1	0.2 ± 0.01
T2 N	-	-	-	-
T1 ALC	-	-	-	-
Unknown 1	-	-	-	-
Polar	0.4 ± 0.3	0.2 ± 0.2	0.8 ± 0.5	0.5 ± 0.2
Aqueous	6.7 ± 0.3	5.0 ± 0.1	4.8 ± 0.1	6.5 ± 0.5

Table 4-24 continued.

	Sulfoxide Citron Control			
Compound	8 [a]	13	20	27
T1	90.1 ± 2.1[b]	85.4 ± 3.7	83.9 ± 0.7	86.1 ± 1.2
T2	0.3 ± 0.2	0.7 ± 0.1	-	0.6 ± 0.3
T1 O	1.1 ± 1.0	1.7 ± 0.1	0.3 ± 0.4	2.7 ± 0.3
T1 AM	-[c]	0.1 ± 0.04	2.8 ± 0.5	0.7 ± 0.7
T2 AM	0.2 ± 0.2	1.6 ± 0.5	-	0.9 ± 0.1
T1 N	0.5 ± 0.4	3.4 ± 2.8	9.1 ± 0.9	3.5 ± 0.3
T2 N	-	-	-	-
T1 ALC	-	0.1 ± 0.1	-	0.1 ± 0.1
Unknown 1	-	-	-	-
Polar	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Aqueous	7.6 ± 0.3	6.8 ± 0.4	3.7 ± 0.5	5.1 ± 0.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Figure 4-3. Percentage of parent sulfoxide and major metabolites in citron callus and control. Sulfoxide in control (*), sulfoxide in tissue flask (●) and sulfoxide oxime + sulfoxide nitrile + sulfone in tissue flask (■).

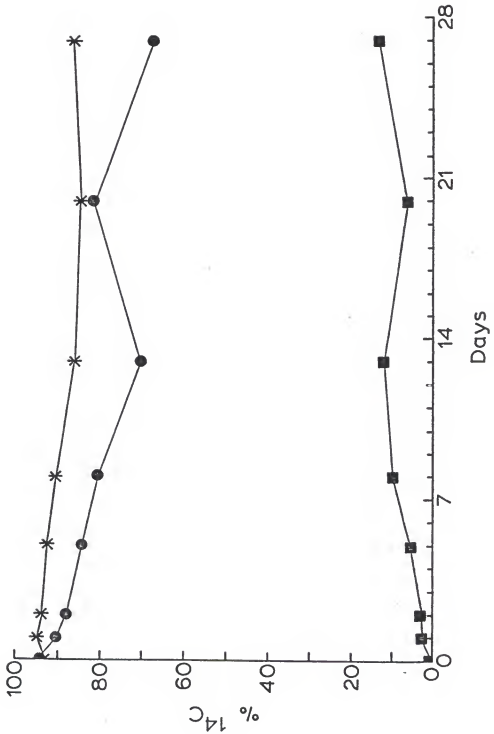

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Figure 4-3 summarizes the metabolic data from application of sulfoxide to citron callus tissue. Sulfoxide was not extensively degraded by the citron callus tissue. The parent sulfoxide percentage decreased slightly with time, and, as shown in the figure, much of the reduction was offset by an increase in the combined percentages of the three major metabolites, the sulfoxide oxime, sulfoxide nitrile and sulfone. Sulfoxide had a calculated half life of 67.9 days (Table 4-12).

The percentage distribution of metabolites, aqueous soluble ^{14}C and bound residue in the whole tuber callus tissue flask is presented in Table 4-25 for the tuber callus sulfoxide experiment. Parent sulfoxide was the major ^{14}C component at all sampling periods and decreased from 90.7% at 0.04 days to 45.0% at 27 days post application. The major metabolites at 27 days were sulfoxide oxime (18.9%) and sulfoxide nitrile (2.4%). Minor metabolites detected were sulfoxide amide, sulfone amide and sulfoxide alcohol, all less than 0.9% each. Aqueous soluble radiocarbon increased from 4.9% at 0.04 days to 30.6% at 27 days. Bound residues were less than 0.3% at all sampling periods.

The metabolite distributions of radiocarbon in tuber callus tissue and in the agar on which it was grown are shown in Tables 4-26 and 4-27 for flasks treated with sulfoxide. Although the percentage of parent sulfoxide was

Table 4-25. Percentage Distribution of Radiocarbon in Tuber Callus Tissues and Agar (Combined) Treated with Aldicarb Sulfoxide.

	Sulfoxide Tuber Tissue and Agar				
Compound	0.04 [a]	1	5	13	27
T1	90.5 ± 0.9[b]	87.3 ± 0.2	77.1 ± 3.7	66.7 ± 3.5	45.0 ± 4.0
T2	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	1.7 ± 0.5	1.0 ± 0.3
T1 0	1.8 ± 1.3	2.4 ± 0.2	3.5 ± 0.6	9.9 ± 1.5	18.9 ± 3.1
T1 AM	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
T2 AM	0.1 ± 0.1	-	-	0.4 ± 0.03	0.2 ± 0.1
T1 N	0.8 ± 0.6	1.3 ± 0.1	0.9 ± 0.3	0.3 ± 0.1	2.4 ± 0.2
T2 N	-[c]	-	-	-	-
T1 ALC	0.03 ± 0.04	-	0.4 ± 0.3	0.02 ± 0.02	0.4 ± 0.2
Unknown 1	-	-	-	-	-
Polar	1.5 ± 1.8	0.6 ± 0.2	0.6 ± 0.3	0.2 ± 0.03	0.4 ± 0.2
Aqueous	4.9 ± 0.2	7.7 ± 0.6	16.5 ± 1.2	20.2 ± 2.1	30.6 ± 3.6
Bound	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.1 ± 0.1	0.3 ± 0.02

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-26. Percentage Distribution of Radiocarbon in Tuber Callus Tissues Treated with Aldicarb Sulfoxide.

	Sulfoxide Tuber Tissue				
Compound	0.04 [a]	1	5	13	27
T1	90.9 ± 1.0[b]	87.5 ± 1.1	65.2 ± 6.2	37.4 ± 1.7	20.7 ± 2.0
T2	0.1 ± 0.1	0.5 ± 0.03	0.8 ± 0.1	2.3 ± 0.4	2.0 ± 0.8
T1 0	1.8 ± 1.3	4.2 ± 0.2	10.7 ± 1.3	15.6 ± 2.3	21.0 ± 2.8
T1 AM	0.2 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.5 ± 0.1
T2 AM	0.1 ± 0.1	-	-	-	0.4 ± 0.3
T1 N	0.7 ± 0.6	2.1 ± 0.3	2.1 ± 0.2	0.6 ± 0.3	1.1 ± 0.5
T2 N	-[c]	-	-	-	-
T1 ALC	0.03 ± 0.04	-	-	-	0.5 ± 0.5
Unknown 1	-	-	-	-	-
Polar	1.4 ± 1.7	0.5 ± 0.5	0.7 ± 0.3	0.4 ± 0.1	0.2 ± 0.1
Aqueous	4.8 ± 0.2	4.8 ± 0.5	19.6 ± 6.0	42.6 ± 1.1	53.0 ± 3.2
Bound	0.01 ± 0.01	0.04 ± 0.02	0.1 ± 0.1	0.5 ± 0.3	0.6 ± 0.1

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-27. Percentage Distribution of Radiocarbon in Agar on which Tuber Callus Tissues were Grown and Treated with Aldicarb Sulfoxide.

	Sulfoxide Tuber Agar				
Compound	0.04 [a]	1	5	13	27
T1	85.7 ± 5.2[b]	86.6 ± 1.5	81.6 ± 1.2	74.2 ± 2.2	62.7 ± 2.5
T2	0.6 ± 0.9	0.6 ± 0.5	0.5 ± 0.04	1.6 ± 0.6	0.4 ± 0.03
T1 0	1.9 ± 2.7	0.7 ± 0.2	2.4 ± 0.5	8.5 ± 1.2	17.3 ± 3.3
T1 AM	-[c]	-	0.5 ± 0.2	0.5 ± 0.1	1.1 ± 0.3
T2 AM	-	-	0.6 ± 0.3	0.5 ± 0.1	-
T1 N	1.4 ± 2.0	0.5 ± 0.2	0.7 ± 0.2	0.2 ± 0.1	3.3 ± 0.02
T2 N	-	-	-	-	-
T1 ALC	-	-	-	0.03 ± 0.03	0.4 ± 0.2
Unknown 1	-	-	-	-	-
Polar	3.4 ± 2.1	0.8 ± 0.7	0.6 ± 0.3	0.1 ± 0.03	0.6 ± 0.4
Aqueous	7.0 ± 1.5	10.8 ± 0.04	13.2 ± 0.7	14.4 ± 0.7	14.2 ± 1.4

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

less in the tissue (20.7%) than in the agar (62.7%), the percentages of identified metabolites in the tissue and agar were similar. The other major difference between the tissue and agar was a higher percentage of aqueous soluble ^{14}C in the tissue (53.0%) than in the agar (14.2%).

The data for the percentage distribution of radiocarbon in the tuber callus control agar treated with the sulfoxide are presented in Table 4-28. The sulfoxide was relatively stable in the control agar with parent percentages ranging between 84.0% and 91.7%. Small amounts (less than 1.0% each) of the sulfone, sulfoxide oxime, sulfoxide nitrile and sulfoxide alcohol were present at 27 days. Aqueous soluble radiocarbon ranged between 6.2% and 10.5%.

Figure 4-4 summarizes the fate of aldicarb sulfoxide in tuber callus tissue. In contrast to citron callus tissue, tuber callus tissue metabolized the sulfoxide to a greater extent. Percentages of aqueous soluble ^{14}C and sulfoxide oxime, when combined almost totally account for reductions of parent sulfoxide with time. The calculated half life of parent sulfoxide in tuber callus tissue was 27.5 days (Table 4-12).

Aldicarb sulfone experiment in citron and tuber callus. The percentage distribution of metabolites, aqueous soluble ^{14}C and unextractable residue in the whole tissue flask are shown in Table 4-29 for the citron callus sulfone experiment. Parent sulfone was the major ^{14}C

Table 4-28. Percentage Distribution of Radiocarbon in Tuber Control Agar Treated With Aldicarb Sulfoxide.

	Sulfoxide Tuber Control				
Compound	0.04 [a]	1	5	13	27
T1	90.0 ± 0.6[b]	86.5 ± 0.7	84.0 ± 3.4	90.3 ± 0.4	91.7 ± 0.9
T2	0.3 ± 0.1	0.4 ± 0.03	2.0 ± 2.4	0.2 ± 0.0	0.2 ± 0.04
T1 0	0.4 ± 0.2	0.7 ± 0.4	0.3 ± 0.04	0.6 ± 0.3	0.6 ± 0.1
T1 AM	-[c]	0.4 ± 0.5	0.4 ± 0.2	-	-
T2 AM	-	-	-	-	-
T1 N	-	0.5 ± 0.3	1.5 ± 1.8	0.2 ± 0.02	0.5 ± 0.01
T2 N	-	-	-	-	-
T1 ALC	-	-	-	-	0.3 ± 0.1
Unknown 1	-	-	-	-	-
Polar	0.3 ± 0.3	1.5 ± 1.0	1.3 ± 0.9	0.1 ± 0.1	0.5 ± 0.1
Aqueous	9.0 ± 0.3	10.1 ± 0.5	10.5 ± 0.6	8.7 ± 0.5	6.2 ± 0.8

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Figure 4-4. Percentage of parent sulfoxide and major metabolites in potato tuber callus and control. Sulfoxide in control (*), sulfoxide in tissue flask (●), sulfoxide oxime in tissue flask (■) and aqueous soluble radiocarbon in tissue flask (▲).

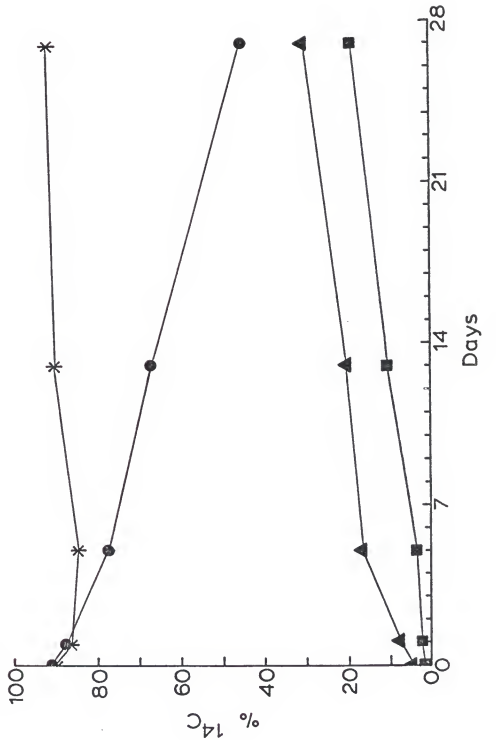

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Table 4-29. Percentage Distribution of Radiocarbon in Citron Callus Tissues and Agar (Combined) Treated with Aldicarb Sulfone.

	Sulfone Citron Tissue and Agar			
Compound	0.04 [a]	1	2	5
T2	91.3 ± 0.4[b]	92.7 ± 0.5	91.7 ± 0.4	88.1 ± 0.9
T2 O	5.5 ± 0.3	5.8 ± 0.1	5.4 ± 0.7	6.5 ± 0.8
T2 AM	-[c]	-	-	-
T2 N	-	-	0.2 ± 0.1	0.3 ± 0.1
Unknown 2	-	0.2 ± 0.2	-	0.6 ± 0.5
Unknown 3	-	-	-	-
Unknown 4	-	-	-	0.2 ± 0.1
Unknown 5	0.8 ± 0.4	0.8 ± 0.4	0.7 ± 0.1	1.3 ± 0.04
Polar	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Aqueous	2.0 ± 0.2	0.3 ± 0.1	1.6 ± 0.2	2.3 ± 0.3
Bound	0.1 ± 0.02	0.1 ± 0.03	0.3 ± 0.1	0.5 ± 0.2

Table 4-29 continued.

Compound	Sulfone Citron Tissue and Agar			
	8 [a]	13	20	27
T2	79.7 ± 2.0[b]	78.7 ± 1.4	73.8 ± 2.0	68.2 ± 3.5
T2 O	6.4 ± 1.4	4.7 ± 2.0	2.4 ± 1.7	2.1 ± 1.1
T2 AM	-[c]	-	3.3 ± 1.3	3.0 ± 0.8
T2 N	-	-	-	-
Unknown 2	0.9 ± 0.3	0.4 ± 0.03	0.7 ± 0.4	1.0 ± 0.2
Unknown 3	-	-	0.2 ± 0.02	1.0 ± 0.6
Unknown 4	1.4 ± 0.2	1.5 ± 0.4	1.0 ± 0.02	1.6 ± 1.0
Unknown 5	2.2 ± 0.5	2.6 ± 0.6	4.0 ± 0.7	3.2 ± 0.3
Polar	0.7 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	1.0 ± 0.2
Aqueous	6.7 ± 1.0	8.6 ± 0.4	11.1 ± 2.0	16.1 ± 3.4
Bound	2.0 ± 0.6	2.7 ± 0.9	3.0 ± 1.3	2.8 ± 0.7

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

component at all sampling periods and decreased from 91.5% at 0.04 days to 68.2% at 27 days. Aqueous soluble ^{14}C increased from 2.0% at 0.04 days to 16.1% at 27 days. The percentage of sulfone oxime remained between 2.0% and 6.5% throughout much of the incubation period. An unidentified compound, designated unknown 4, represented 3.2% to 4.0% of the ^{14}C in the citron tissue flask by 20 to 27 days. The sulfone amide was detected at 20 days (approximately 3.0%). Three other unknowns were present at low levels at the later sampling periods. The bound residue increased throughout the sampling period to a maximum value of 2.8%.

The data showing percentages of metabolites in the citron callus tissue and agar on which it was grown are presented in Tables 4-30 and 4-31, respectively. The percentage of parent sulfone was lower in the tissue than in the agar at 27 days, 41.9% and 77.4%, respectively. Percentages of the sulfone oxime, amide and unknowns were also higher in the agar. Percentages of aqueous soluble ^{14}C were 40.5% and 7.6% at 27 days in the tissue and agar, respectively. The percentages of bound residue of the tissue radiocarbon increased by the last four sampling periods from 9.4% to 13.9%.

The percentage distribution of radiocarbon in the citron sulfone experiment control agar is shown in Table 4-32. Parent sulfone was stable throughout the sampling period ranging between 91.1% to 93.5%. Five to 6.0%

Table 4-30. Percentage Distribution of Radiocarbon in Citron Callus Tissues Treated with Aldicarb Sulfone.

	Sulfone Citron Tissue			
Compound	0.04[a]	1	2	5
T2	91.5 ± 0.9[b]	93.2 ± 0.8	91.7 ± 1.0	86.1 ± 2.7
T2 O	5.4 ± 0.3	5.4 ± 0.4	4.7 ± 0.8	6.2 ± 1.4
T2 AM	-[c]	-	-	-
T2 N	-	-	-	0.4 ± 0.1
Unknown 2	-	0.3 ± 0.3	-	1.3 ± 1.2
Unknown 3	-	-	-	-
Unknown 4	-	-	-	-
Unknown 5	0.7 ± 0.7	0.5 ± 0.3	0.6 ± 0.02	0.9 ± 0.2
Polar	0.2 ± 0.3	0.1 ± 0.04	0.3 ± 0.4	0.1 ± 0.2
Aqueous	2.1 ± 0.04	0.2 ± 0.0	2.1 ± 0.1	3.6 ± 0.7
Bound	0.1 ± 0.03	0.3 ± 0.1	0.6 ± 0.1	1.5 ± 0.9

Table 4-30 continued.

Compound	Sulfone Citron Tissue			
	8[a]	13	20	27
T2	69.0 ± 3.0[b]	55.3 ± 3.6	49.8 ± 2.9	41.8 ± 10.3
T2 O	2.2 ± 0.6	1.6 ± 0.7	2.0 ± 1.7	0.9 ± 0.2
T2 AM	-[c]	-	2.7 ± 0.8	1.7 ± 0.8
T2 N	-	-	-	-
Unknown 2	2.1 ± 0.9	-	1.2 ± 1.2	1.3 ± 0.3
Unknown 3	-	-	0.2 ± 0.2	-
Unknown 4	-	-	-	-
Unknown 5	-	-	1.9 ± 0.2	2.0 ± 0.5
Polar	1.9 ± 0.7	2.4 ± 0.4	0.9 ± 0.7	2.1 ± 0.4
Aqueous	15.4 ± 2.7	27.1 ± 2.9	27.4 ± 2.4	40.5 ± 13.5
Bound	9.4 ± 3.0	13.6 ± 4.5	13.9 ± 5.4	9.7 ± 4.2

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-31. Percentage Distribution of Radiocarbon in Agar on which Citron Callus Tissues were Grown and Treated with Aldicarb Sulfone.

	Sulfone Citron Agar			
Compound	0.04[a]	1	2	5
T2	89.4 ± 2.0[b]	91.7 ± 1.0	91.1 ± 0.6	89.0 ± 1.0
T2 O	5.2 ± 0.6	6.1 ± 0.2	6.1 ± 0.1	6.7 ± 0.7
T2 AM	-[c]	-	-	-
T2 N	-	-	0.4 ± 0.1	0.4 ± 0.04
Unknown 2	-	-	-	0.3 ± 0.1
Unknown 3	-	-	-	-
Unknown 4	-	-	-	0.4 ± 0.2
Unknown 5	-	1.5 ± 0.8	1.0 ± 0.4	1.6 ± 0.3
Polar	1.5 ± 1.3	0.2 ± 0.1	-	0.2 ± 0.1
Aqueous	3.9 ± 0.1	0.5 ± 0.1	1.2 ± 0.2	1.4 ± 0.3

Table 4-31 continued.

	Sulfone Citron Agar			
Compound	8 [a]	13	20	27
T2	82.6 ± 2.5[b]	84.4 ± 2.1	80.2 ± 1.3	77.3 ± 1.6
T2 0	7.6 ± 1.7	5.5 ± 2.4	2.6 ± 1.7	2.9 ± 2.0
T2 AM	-[c]	-	3.5 ± 1.5	3.6 ± 0.9
T2 N	-	-	-	-
Unknown 2	0.5 ± 0.2	0.5 ± 0.02	0.6 ± 0.3	0.9 ± 0.2
Unknown 3	-	-	0.2 ± 0.04	1.4 ± 0.8
Unknown 4	1.8 ± 0.3	1.8 ± 0.5	1.3 ± 0.1	2.1 ± 1.0
Unknown 5	-	3.2 ± 0.8	4.6 ± 0.8	3.6 ± 0.5
Polar	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.6 ± 0.1
Aqueous	7.2 ± 0.8	4.2 ± 0.7	6.6 ± 1.0	7.6 ± 0.7

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-32. Percentage Distribution of Radiocarbon in Citron Control Agar Treated with Aldicarb Sulfone.

	Sulfone Citron Control			
Compound	0.04 –[a]	1	2	5
T2	92.2 ± 0.2[b]	93.5 ± 0.6	91.8 ± 0.7	92.6 ± 0.4
T2 O	5.9 ± 0.1	5.0 ± 0.3	5.5 ± 0.2	5.4 ± 0.2
T2 AM	–[c]	-	-	0.2 ± 0.04
T2 N	-	0.2 ± 0.1	0.3 ± 0.04	0.3 ± 0.1
Unknown 2	-	-	-	-
Unknown 3	-	-	-	-
Unknown 4	-	-	-	-
Unknown 5	0.9 ± 0.03	0.6 ± 0.3	0.5 ± 0.1	0.3 ± 0.1
Polar	0.3 ± 0.1	0.3 ± 0.3	0.4 ± 0.4	0.4 ± 0.2
Aqueous	0.7 ± 0.1	0.4 ± 0.01	1.5 ± 0.1	0.8 ± 0.1

Table 4-32 continued.

	Sulfone Citron Control			
Compound	8 [a]	13	20	27
T2	91.4 ± 0.9[b]	92.2 ± 0.9	91.1 ± 0.5	91.6 ± 0.9
T2 0	5.8 ± 0.1	5.1 ± 0.1	5.3 ± 0.3	5.3 ± 0.3
T2 AM	-[c]	-	0.8 ± 0.1	0.4 ± 0.2
T2 N	-	-	-	-
Unknown 2	-	-	-	
Unknown 3	-	-	-	-
Unknown 4	0.3 ± 0.3	0.5 ± 0.4	0.8 ± 0.1	0.7 ± 0.2
Unknown 5	0.7 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.2
Polar	1.0 ± 0.7	0.4 ± 0.3	-	0.4 ± 0.3
Aqueous	0.8 ± 0.5	1.2 ± 0.3	1.5 ± 0.3	1.1 ± 0.7

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

sulfone oxime was detected at all sampling periods and the percentages of all other conversion products detected were below 1.0%. Aqueous soluble ^{14}C represented between 0.4% to 1.5% of the radiocarbon.

Figure 4-5 summarizes the data when sulfone was applied to citron callus tissues. The percentage of parent sulfone in the control remained at approximately 95% throughout the incubation period. The half life of sulfone in citron callus tissue was 60.6 days (Table 4-12).

The percentage distribution of metabolites, aqueous soluble ^{14}C and bound residue in the whole tissue flask is presented in Table 4-33 for the tuber callus tissue sulfone experiment. Parent sulfone was present at all post application sampling periods and the percentage had decreased significantly by 27 days to 15.2%. The major identified metabolite, the sulfone oxime, increased in percentage to 42.7% at 27 days. Aqueous soluble ^{14}C increased in percentage from 0.2% at 0.04 days to 38.2% at 27 days. Bound residues were less than 2.0% at all sampling periods.

The metabolite distributions of ^{14}C in the tuber callus tissue and agar on which it was grown are shown in Tables 4-34 and 4-35. As in the aldicarb and sulfoxide citron and tuber callus tissue experiments the percentage of parent compound was lower in the tissue than in the agar. In this case the percentage of parent sulfone at 27

Figure 4-5. Percentage of parent sulfone and major metabolites in citron callus and control. Sulfone in control (*), sulfone in tissue flask (●) and aqueous soluble radiocarbon in tissue flask (■).

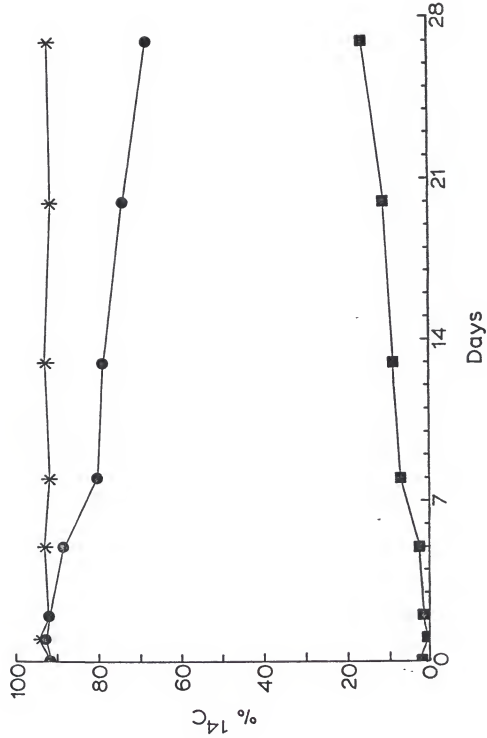

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Table 4-33. Percentage Distribution of Radiocarbon in Tuber Callus Tissues and Agar (Combined) Treated with Aldicarb Sulfone.

	Sulfone Tuber Tissue and Agar				
Compound	0.04 [a]	1	5	13	27
T2	79.1 ± 9.1[b]	94.0 ± 0.4	82.9 ± 1.9	63.6 ± 10.3	15.5 ± 11.2
T2 O	12.3 ± 6.0	3.8 ± 0.4	7.4 ± 2.0	19.1 ± 5.3	42.7 ± 0.3
T2 AM	-[c]	0.1 ± 0.03	0.2 ± 0.1	0.5 ± 0.2	-
T2 N	-	-	-	-	0.8 ± 0.1
Unknown 2	-	0.1 ± 0.02	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.2
Unknown 3	-	-	-	-	-
Unknown 4	-	-	0.8 ± 0.6	1.2 ± 0.3	-
Unknown 5	4.3 ± 2.1	0.3 ± 0.04	0.4 ± 0.1	0.7 ± 0.1	1.7 ± 0.1
Polar	4.1 ± 2.3	0.1 ± 0.03	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.3
Aqueous	0.2 ± 0.1	1.5 ± 0.3	6.8 ± 0.7	12.9 ± 5.1	37.1 ± 11.0
Bound	0.0 0.0	0.1 0.01	0.5 0.2	0.9 0.2	1.1 0.1

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-34. Percentage Distribution of Radiocarbon in Tuber Callus Tissues Treated with Aldicarb Sulfone.

	Sulfone Tuber Tissue				
Compound	0.04 [a]	1	5	13	27
T2	79.0 ± 9.1[b]	93.7 ± 0.6	63.2 ± 4.3	23.3 ± 7.5	3.9 ± 1.2
T2 0	12.5 ± 6.1	4.3 ± 0.2	14.9 ± 2.3	23.9 ± 3.7	33.3 ± 4.3
T2 AM	-[c]	0.3 ± 0.1	-	0.6 ± 0.4	-
T2 N	-	-	-	-	0.6 ± 0.1
Unknown 2	-	0.1 ± 0.1	0.3 ± 0.04	0.3 ± 0.2	0.4 ± 0.5
Unknown 3	-	-	-	-	-
Unknown 4	-	-	0.3 ± 0.2	-	-
Unknown 5	4.3 ± 2.1	0.4 ± 0.01	0.6 ± 0.2	2.2 ± 1.4	1.5 ± 0.1
Polar	4.1 ± 2.3	0.2 ± 0.1	1.1 ± 0.4	3.8 ± 2.1	1.1 ± 0.4
Aqueous	0.1 ± 0.02	0.9 ± 0.4	14.8 ± 3.3	39.9 ± 9.9	56.8 ± 1.4
Bound	0.01 ± 0.1	0.1 ± 0.1	4.8 ± 1.0	6.0 ± 3.0	2.4 ± 1.0

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Table 4-35. Percentage Distribution of Radiocarbon in Agar on which Tuber Callus Tissues were Grown and Treated with Aldicarb Sulfone.

	Sulfone Tuber Agar				
Compound	0.04 [a]	1	5	13	27
T2	86.1 ± 8.1[b]	94.0 ± 0.3	84.9 ± 1.5	73.8 ± 5.7	24.5 ± 11.2
T2 0	-[c]	3.6 ± 0.7	6.6 ± 1.8	18.0 ± 5.2	54.1 ± 4.4
T2 AM	-	-	0.3 ± 0.1	0.4 ± 0.1	-
T2 N	-	-	-	-	1.1 ± 0.1
Unknown 2	-	-	0.4 ± 0.3	0.3 ± 0.1	0.4 ± 0.1
Unknown 3	-	-	-	-	-
Unknown 4	-	-	0.9 ± 0.6	1.5 ± 0.4	-
Unknown 5	-	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	2.0 ± 0.2
Polar	4.2 ± 4.8	0.02 ± 0.02	0.5 ± 0.3	0.3 ± 0.2	0.8 ± 0.4
Aqueous	9.7 ± 5.4	2.1 ± 0.5	6.0 ± 0.6	5.2 ± 1.4	17.1 ± 6.3

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

days was 3.9% in the tuber callus tissue and 24.6% in the agar. The percentages of some other metabolites were also higher in the agar than in the tissue. For example, sulfone oxime represented 33.3% in the tissue compared to 54.14% in the agar. This difference was accounted for by a higher percentage of aqueous ^{14}C in the tissue than the agar, 56.8% to 17.1% at 27 days. The percentage of bound residue in the tuber callus tissue reached a maximum value of 6.0% at the 13 days interval. This was also when the percentage of total tissue ^{14}C was lowest.

The percentage distribution of radiocarbon in the sulfone tuber control agar is shown in Table 4-36. The percentage of parent sulfone ranged between 94.4% and 98.3% throughout the sampling periods. The percentage of aqueous soluble ^{14}C ranged between 0.7% and 4.3%. There were only small amounts (less than 0.7%) of other conversion products detected.

Figure 4-6 summarizes the data from application of sulfone to tuber callus tissues. The percentage of parent sulfone in the control remained above 95% throughout the sampling periods. Along with the almost linear decrease in parent sulfone there was an equivalent increase in the combined percentages of sulfone oxime and aqueous soluble radiocarbon. The percentages of sulfone oxime and aqueous ^{14}C were almost equivalent throughout the sampling periods. Again, the aqueous material was most likely a mixture of

Table 4-36. Percentage Distribution of Radiocarbon in Tuber Control Agar Treated with Aldicarb Sulfone.

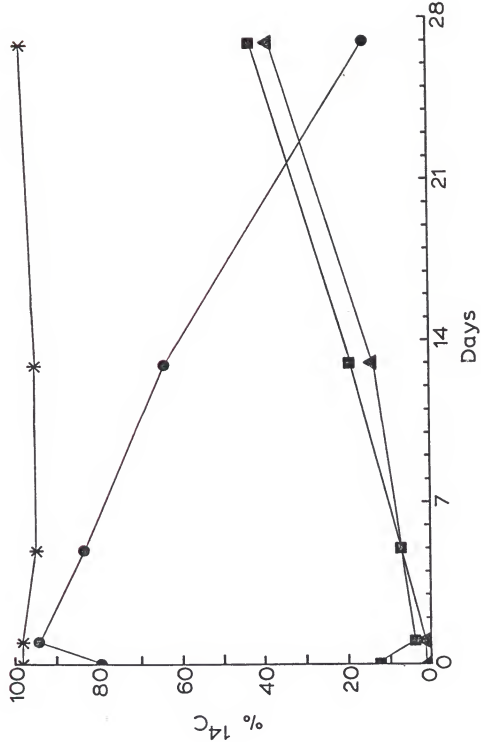
	Sulfone Tuber Control				
Compound	0.04 [a]	1	5	13	27
T2	98.3 ± 0.5[b]	98.1 ± 0.4	94.4 ± 0.7	94.3 ± 0.4	97.5 ± 0.3
T2 O	-[c]	0.2 ± 0.02	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.04
T2 AM	-	-	-	-	-
T2 N	-	-	-	-	0.3 ± 0.1
Unknown 2	-	-	-	-	-
Unknown 3	-	-	-	-	-
Unknown 4	-	-	-	-	0.3 ± 0.1
Unknown 5	-	-	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Polar	0.1 ± 0.1	0.04 ± 0.01	0.5 ± 0.3	0.6 ± 0.1	0.2 ± 0.01
Aqueous	1.6 ± 0.5	1.7 ± 0.4	4.3 ± 0.3	3.9 ± 0.4	0.7 ± 0.2

[a] Days post application.

[b] Mean of triplicate samples and standard deviation.

[c] None detected.

Figure 4-6. Percentage of parent sulfone and major metabolites in potato tuber callus and control. Sulfone in control (*), sulfone in tissue flask (●), sulfone oxime in tissue flask (■) and aqueous soluble radiocarbon in tissue flask (▲).


% 14C
100
80
60
40
20
0
0
7
14
21
28
Days

conjugates of decarbamylated products and free sulfone acid. The half life of sulfone in tuber callus tissue was 10.9 days (Table 4-12).

Discussion

Comparisons of the percentage decreases for a given parent compound in the two callus systems are made in Figures 4-7, 4-8 and 4-9. The data in these figures are the percentages from the whole tissue flask (i.e. tissue ^{14}C combined with agar ^{14}C). The control in each case is the average of the controls from the tuber callus experiment and the citron callus experiment. These figures and data previously described will be used to explain some of the metabolic events which occur in the tuber and citron callus tissue culture systems.

Figure 4-7 shows the percentages of parent aldicarb in tuber callus tissue and citron callus tissue throughout the sampling periods. Both callus systems degraded aldicarb extensively. There were, however, some major differences in the rates of degradation and the products. The pseudo first order rate constants (k), for the tuber and citron callus systems were 0.100 and 0.247, respectively. The half-life of aldicarb in citron callus (1.0 day) was much shorter than in tuber callus (6.9 days) under these particular conditions. Figure 4-8 shows the percentages of parent sulfoxide in both callus tissue systems. In contrast to the aldicarb experiments, the sulfoxide

Figure 4-7. Percentage of parent aldicarb in citron and potato tuber callus and control. Aldicarb in control (*), aldicarb in citron tissue flask (■) and aldicarb in tuber tissue flask (●).

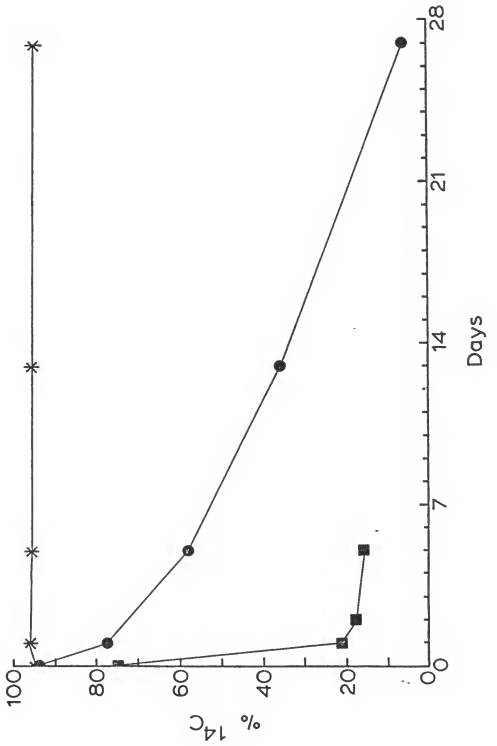

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Figure 4-8. Percentage of parent sulfoxide in citron and potato tuber callus and control. Sulfoxide in control (*), sulfoxide in citron callus (■) and sulfoxide in tuber callus (●).

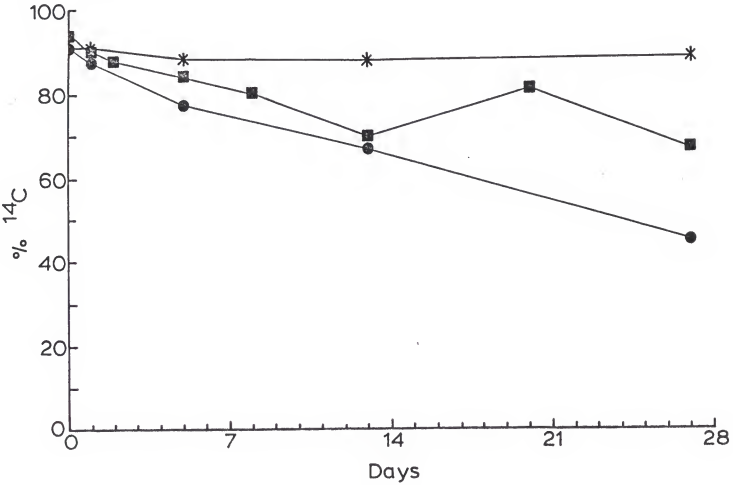
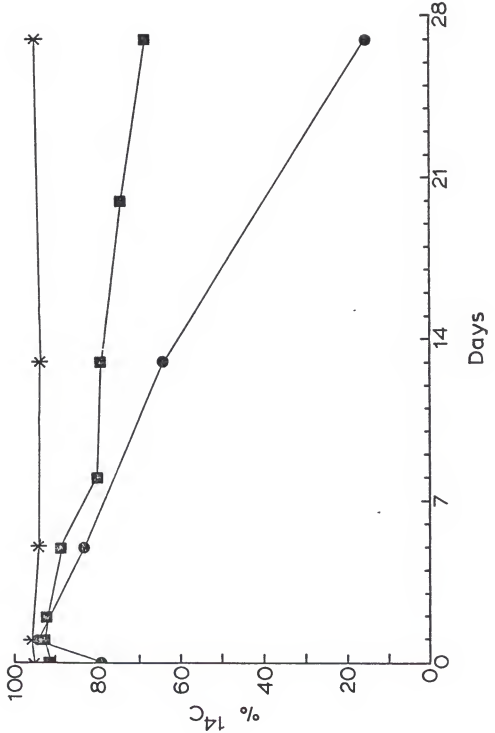

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Figure 4-9. Percentage of parent sulfone in citron and potato tuber callus and control. Sulfone in control (*), sulfone in citron callus (■) and sulfone in tuber callus (●).


100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

degradation rates were slower, k = 0.025 and 0.010 for tuber and citron callus, respectively. Also, tuber callus caused a faster degradation of the sulfoxide. Half lives were 27.5 and 67.9 days for the tuber and citron callus, respectively. Figure 4-9 shows the percentages of parent sulfone in the two callus tissue systems. The pseudo first order rate constant was 0.064 in tuber callus and 0.011 in citron callus. Tuber callus, as in the sulfoxide experiment, was more effective at degrading sulfone with a half-life of 10.9 days compared to 60.6 days for citron callus.

In the citron callus the decrease in percentage of aldicarb was accompanied by an almost equivalent increase in the percentage of sulfoxide (Figure 4-1 and Table 4-13). A single sulfoxidation was the major metabolic activity since very little sulfone and aqueous soluble ^{14}C was formed. Aqueous soluble ^{14}C was not analyzed in these experiments, however, data from Chapter III and the literature suggest that water soluble materials derived from aldicarb were typically decarbamylated products such as sulfoxide and sulfone alcohol, sulfoxide and sulfone amide and sulfoxide and sulfone acid, existing primarily as conjugates (the acids also appear as free aqueous soluble species). Therefore, in the aldicarb citron experiment, once sulfoxide was formed there was very little decarbamylation by 5 days. In the aldicarb tuber callus experiment the percentage decrease in parent aldicarb was

accompanied by an almost equivalent increase in the percentage of aqueous soluble ^{14}C (Figure 4-2 and Table 4-17) rather than an increase in the percentage of sulfoxide. The data suggest that in the tuber callus tissue there was major metabolic activity for decarbamylation. These products could possibly occur from a combination of events involving decarbamylation of aldicarb, and sulfoxidation of aldicarb to the sulfoxide followed by decarbamylation.

The results from sulfoxide and sulfone experiments with tuber and citron callus tissues can be used to help substantiate and clarify the overall metabolism of aldicarb. In the sulfoxide citron and tuber callus experiments, sulfoxide was not degraded as extensively as aldicarb. Citron callus oxidized sulfoxide to sulfone (3.0%), decarbamylated products (sulfoxide amide (4.8%), sulfoxide oxime (3.0%), sulfoxide nitrile (6.7%)) and to aqueous soluble ^{14}C (11.3%). At 27 days (Table 4-21), parent sulfoxide still represented 67% of the radiocarbon. This indicates small amounts of oxidation to sulfone and larger amounts of decarbamylation. Conjugation of the decarbamylated products does not appear to be a major pathway since only 11.3% aqueous soluble ^{14}C was formed. In contrast, at 27 days in tuber callus the sulfoxide percentage was 45.0% and only 1.0% sulfone was detected. Decarbamylation to sulfoxide oxime (18.9%) and aqueous soluble material (30.57%) (Table 4-17) indicates a higher

level of conjugate formation, and possibly free acid formation. These data correspond to that observed with aldicarb in the two callus systems. Figure 4-10 shows a proposed scheme for the metabolism of aldicarb and sulfoxide in citron callus and tuber callus.

As with the other two compounds, aldicarb sulfone was more rapidly degraded by tuber callus than citron callus. In the citron callus most of the percentage decrease by 27 days in parent was accompanied by an increase in aqueous soluble ^{14}C and small amounts of sulfone oxime (2.1%) sulfone amide (3.0%) and unidentified compounds. By 27 days, tuber callus, however, had reduced parent sulfone to 15.2% and this decrease was accompanied by increases in hydrolysis products, sulfone oxime (42.7%) and aqueous soluble ^{14}C (38.2%). Figure 4-11 shows a proposed summary of the metabolic pathway of sulfone in citron and tuber callus tissue culture.

To summarize, there appeared to be no major differences qualitatively in the metabolites formed from application of aldicarb, aldicarb sulfoxide and aldicarb sulfone to citron and tuber callus tissues. The major difference was in the quantitative distribution of metabolites. Tuber callus generally yielded a higher percentage of hydrolysis products which led to formation of conjugates than did citron callus. Also, the relatively high percentages of sulfoxide oxime present in the aldicarb and sulfoxide

Figure 4-10 Proposed metabolic pathway of aldicarb and aldicarb sulfoxide in potato tuber and citron fruit callus tissue.

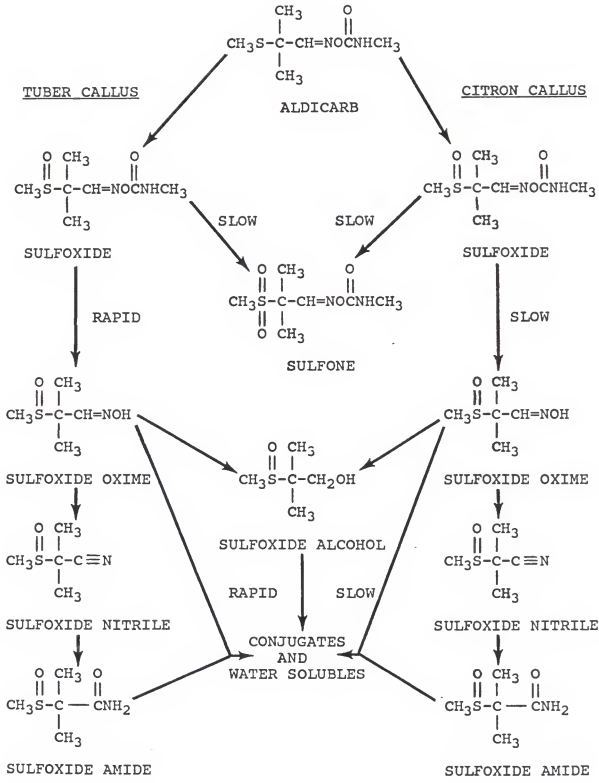
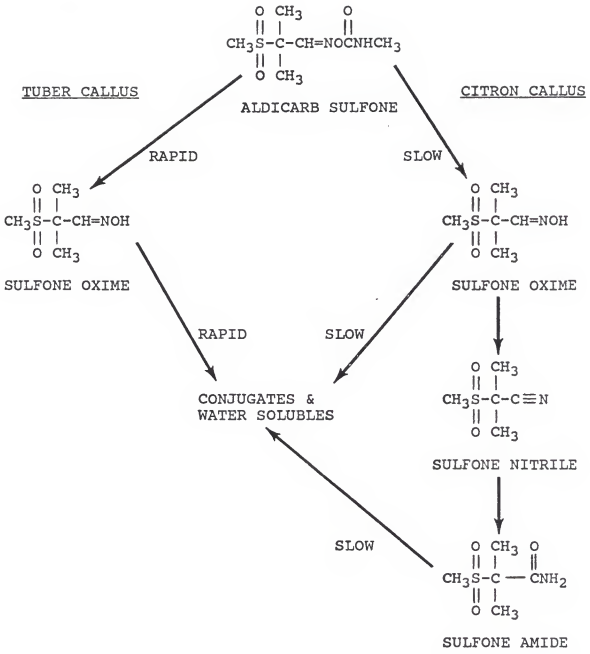

TUBER CALLUS
ALDICARB
CITRON CALLUS
SLOW
SLOW
SULFOXIDE
SULFOXIDE
SULFONE
RAPID
SLOW
SULFOXIDE OXIME
SULFOXIDE ALCOHOL
SULFOXIDE OXIME
SULFOXIDE NITRILE
SULFOXIDE NITRILE
RAPID
SLOW
CONJUGATES
AND
WATER SOLUBLES
SULFOXIDE AMIDE
SULFOXIDE AMIDE

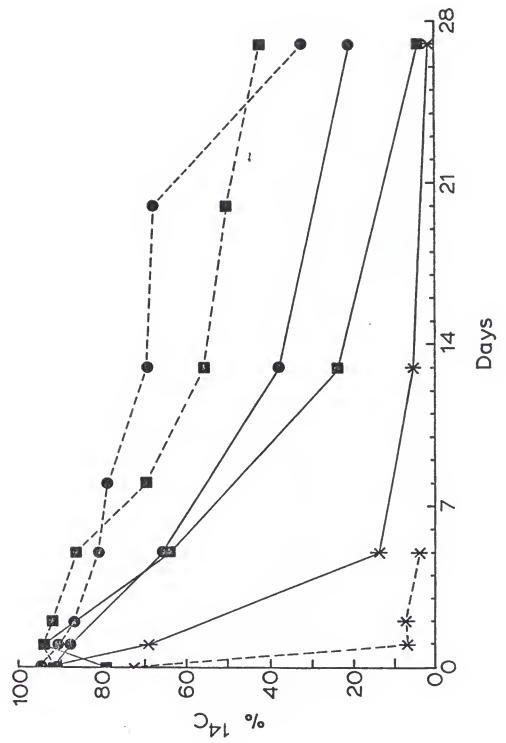
Figure 4-11. Proposed metabolic pathway of aldicarb sulfone in potato tuber and citron fruit callus tissue.


ALDICARB SULFONE
TUBER CALLUS
CITRON CALLUS
RAPID
SLOW
SULFONE OXIME
SULFONE OXIME
RAPID
SLOW
CONJUGATES &
WATER SOLUBLES
SULFONE NITRILE
SLOW
SULFONE AMIDE

experiments and sulfone oxime in the sulfone experiment suggest that conjugation of the oximes was not a major pathway. Rather, metabolism of the oximes to corresponding alcohols and amides, which then formed conjugates, appeared to predominate.

The above discussion concerns data calculated from the total radiocarbon content of the whole tissue flask (e.g. agar ^{14}C and tissue ^{14}C combined). This was presented in this manner so that all the radiocarbon in the sample flasks could be accounted for and so that the citron and tuber callus datacould be directly compared, since there were significant differences in the percentages of radiocarbon in only the tissues of the two callus tissue types at the various sampling periods. Another perspective of the metabolism of these compounds in the callus tissues can be obtained by evaluating only the radiocarbon content of the callus tissue. All the trends described above still exist when this is done. There is generally a greater percentage of the parent degraded by 27 days for all three compounds in both callus tissues. Figure 4-12 shows a comparison of the percentage of parent aldicarb, sulfoxide and sulfone in tissues of both callus systems. It is evident from this figure that tuber callus tissue degraded the parent compounds more rapidly and to a greater extent under these experimental conditions, with one exception for

Figure 4-12. Percentage of parent aldicarb, sulfoxide and sulfone in only tissues of citron and potato tuber callus. Aldicarb in citron tissue (--*--), aldicarb in tuber tissue (*), sulfoxide in citron tissue (--●--), sulfoxide in tuber tissue (●), sulfone in citron tissue (--■--) and sulfone in tuber tissue (■).


100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

aldicarb. At 5 days the percentage of parent aldicarb was lower in the citron callus than in the tuber callus.

Analysis of the tissue and agar separately allowed a quantitative determination of the dynamics of movement and distribution of radiocarbon between the tissue and agar at the specific sampling dates. The test compounds were applied to the callus tissue in such a way that all of the compound could be absorbed by the tissue. Then transfer of the radiocarbon would occur by diffusion through the callus tissue and/or by an active transport process to the agar. The percentages of radiocarbon in the citron and tuber callus tissue at the various sampling periods for the three compounds (Tables 4-8 and 4-10) are plotted in Figures 4-13 and 4-14, respectively. In both types of callus tissue and for all three compounds there was a very rapid movement of radiocarbon from the tissue to the agar. By 1 day post application approximately 50% or more of the radiocarbon had moved into the agar in all the experiments except for the sulfoxide in the citron callus tissue experiment. In the tuber callus experiment there was a very consistent movement of ^{14}C out of the tissue at early sampling periods (0.04 to 5 days) and then movement back into the tissue at the later sampling periods. With citron callus, however, the results were somewhat erratic. Aldicarb was monitored only until day 5 and it is not known whether there would be net movement back into the tissue by 27 days. Sulfone

Figure 4-13. Percentage of total radiocarbon in citron callus tissues treated with aldicarb, sulfoxide and sulfone. ^{14}C in aldicarb treated tissues (*), ^{14}C in sulfoxide treated tissues (●) and ^{14}C in sulfone treated tissues (■).

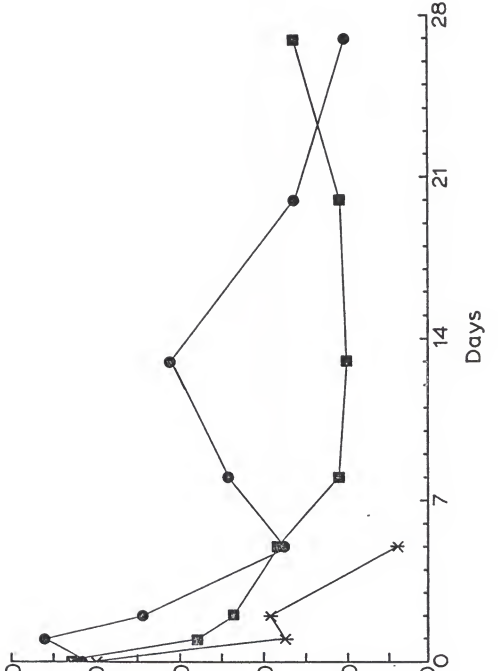
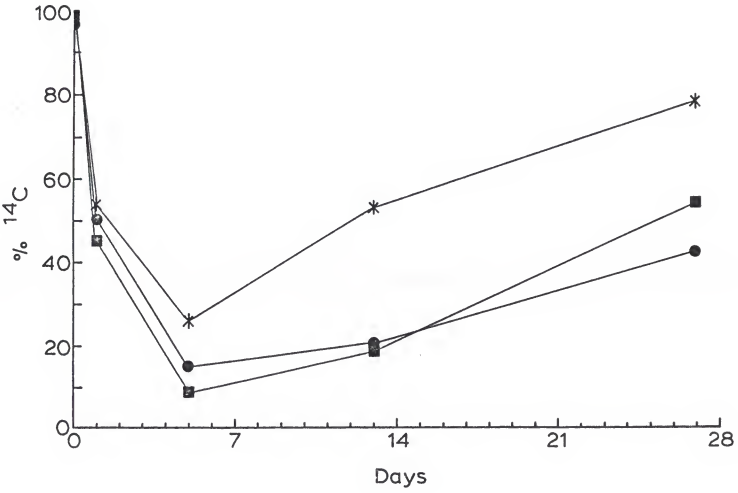

100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

Figure 4-14. Percentage of total radiocarbon in potato tuber callus treated with aldicarb, sulfoxide and sulfone. ^{14}C in aldicarb treated tissues (*), ^{14}C in sulfoxide treated tissues (●) and ^{14}C in sulfone treated tissues (■).


100
80
60
40
20
0
% 14C
0
7
14
21
28
Days

showed a decline in tissue ^{14}C throughout sampling until 27 days when a slight increase was detected. Sulfoxide showed a peak in tissue ^{14}C at 13 days which had decreased by 27 days. One possible explanation for increases in tissue ^{14}C in the tuber callus experiment at the later sampling periods, which did not occur in citron callus, was that the tuber callus was a much more friable and rapidly growing callus tissue than citron callus (Tables 4-2 and 4-3). By 27 days tuber callus tissue weights (total tissue weight in flask) averaged approximately 15 to 20 g compared to 3.5 to 5.0 g for the citron callus. Data from individual samples for both tuber and citron tissues indicated that there was some correlation between the tissue weight and the percentage of the applied ^{14}C in the tissue. This correlation was apparent, however, only when the differences in weight were large. Therefore, the tissue weight differences were reflected in the standard deviations of average tissue weights (Tables 4-2 and 4-3) and percentage distribution of ^{14}C between agar and tissue (Tables 4-8, 4-9, 4-10 and 4-11).

Although there was some effect of tissue weight on percentage of tissue ^{14}C, there was apparently minimal effect on the percentage distribution of metabolites in a given sample flask. This was the case whether data from the whole tissue flask or only the tissue was considered.

Utilization of plant tissue culture to study the metabolism of pesticides has many advantages over using intact plants grown under growth chamber, greenhouse or field conditions. In studies performed on in vivo plants, workers often have difficulty differentiating between actual metabolic effects by the plant and confounding interaction from external environmental factors such as microbial metabolism and light catalyzed reactions. Tissue cultures can be kept sterile, thus eliminating microbial metabolism and can be grown in the dark to minimize or eliminate light catalyzed transformations. Growth conditions can be easily standardized producing greater reproducibility and the data lend themselves to statistical analysis. However, many published metabolism experiments using plant cell cultures have not reported statistical analysis of the data, according to several review articles on the subject (Mumma and Hamilton, 1979, Sanderman et al., 1977, Mumma and Davidonis, 1983, and Sanderman et al., 1984). Sanderman et al. (1984) used suspension cell culture techniques to study metabolism of hexachlorobenzene (HCB), pentachlorophenol (PCP), diethylhexylphthalate (DEHP), benzo[a]pyrene (BaP) and DDT and to develop a rapid and reliable assay technique. They performed 6 to 12 parallel incubations and obtained relative standard deviations of ± 10% to 20%. These results correspond well

with the standard deviations obtained in the present experiment.

There are many factors which can affect metabolism of xenobiotic chemicals in plant tissue culture. Suspension cultures can yield different results than callus culture experiments. There can be differences in the uptake of the chemical and exchange of the chemical between the media and the cells. This type of exchange is probably related in part to the octanol/water partitioning coefficient of the xenobiotic. Sanderman et al. (1984) found that in suspension cultures that autoclaved cell debris removed significant portions of the radioactivity from the growth medium; autoclaved cell debris actually removed more of the lipophilic compounds than did living cells. Also, they found a wide range of ^{14}C in the liquid media among the various compounds, ranging from less than 10% with HCB, PCP, DEPH and DDT to 86% with 2,4,5-T.

The mode of application, or exposure, of the test compound to the cells or callus tissues can also affect metabolism results. Soybean callus tissues injected with ^{14}C 2,4-D and incubated on solid agar yielded higher levels of metabolites than callus tissues exposed in solution shake cultures (Feung et al., 1978). The injection method was similar to the technique used in the present experiment in which the ^{14}C was pipetted directly onto the callus piece. However, after 8 days 85% of the ^{14}C was in the

soybean callus suggesting there was not much movement of ^{14}C from the callus to the agar. Much less ^{14}C remained in both the citron and tuber tissues in callus experiments reported here. The difference could be a result of differences in the test chemicals and callus tissue types.

There are obvious disadvantages to utilization of metabolism results from tissue culture experiments for extrapolation to the behavior of a chemical in whole plants. The results give no indication of root absorption or foliar penetration after foliar spray. Since they are sterile, there are no normal plant and soil associated microorganisms. Therefore, effects of normal environmental factors on the intact plant are not represented. Also, since many cultures are grown in the dark, photosynthetic mechanisms are not fully operational. Cells in tissue culture also become undifferentiated and thus do not truly represent differentiated cell types in the intact plant. This could lead to physiological differences causing certain metabolic pathways to become inoperative in culture (Gressel, 1980).

In summary, the present experiment with aldicarb, aldicarb sulfoxide and aldicarb sulfone in citron and potato tuber callus tissue cultures was a useful and rapid metabolic assay technique. Good reproducibility was obtained as reflected by the standard deviations observed among the samples. Extensive degradation of the parent

compounds was demonstrated in both callus types. There were quantitative differences in the metabolic distribution between the callus types, however, qualitatively, the types of metabolites found were similar.

CHAPTER V

SUMMARY AND CONCLUSIONS

Aldicarb, aldicarb sulfoxide and aldicarb sulfone were degraded extensively in both whole plant *in vivo* experiments using potato plants and in *in vitro* experiments utilizing callus tissues derived from citron fruit and potato tuber. The metabolism of these three compounds in the intact potato plants was reported in Chapter III; metabolism in the two callus tissue culture systems was reported in Chapter IV where comparisons were made between the two callus types. These experiments provided an excellent opportunity to compare results from *in vivo* and *in vitro* conditions. There were no qualitative differences when considering the types of metabolites and conversion products formed in whole potato plants and in the potato tuber and citron callus systems, but considerable differences were observed in the quantitative aspects of metabolite formation.

In all experiments aldicarb was converted to aldicarb sulfoxide as a major pathway. After application of aldicarb the percentage of the sulfoxide which was present

in the various plant parts of whole potato plants and in the two callus types differed widely at the last sampling period in all experiments. The percentage of sulfoxide was high in the foliage of whole potato plants (41.1%) and in citron callus tissue (67.31%). The percentages were lower in the other plant parts, stem (24.4%), seed (13.7%) and root (2.8%). The percentage of sulfoxide in the tubers of whole plants treated with aldicarb and in callus tissues derived from potato tubers of the same potato species were remarkably similar, 12.9% and 9.7%, respectively. These low percentages of sulfoxide in the in vivo tubers and in the tuber callus were accompanied by high percentages of decarbamylated products such as the sulfoxide and sulfone oximes, alcohols, nitriles and amides. The alcohols and amides were present primarily as conjugates. These products were formed to a lesser extent in potato foliage and to an even lesser extent in citron callus.

Even higher percentages of aldicarb sulfoxide were present in tuber callus at the last sampling period when aldicarb sulfoxide was applied than was the case when aldicarb was applied. Forty-five percent of the parent sulfoxide was present 27 days post application compared to 9.7% at 27 days in the aldicarb experiment. This suggests that aldicarb was rapidly hydrolyzed to decarbamylated products which were then conjugated prior to sulfoxidation. It also suggests that the hydrolysis of aldicarb sulfoxide

was a relatively slow process. In order to evaluate this hypothesis, it would be necessary to detect and quantify the non-oxidized aldicarb metabolites. The data generated from this research did not provide information about these possible compounds. Non-oxidized aldicarb metabolites (e.g. aldicarb oxime) were not detected, possibly as a result of being formed, and then being oxidized. This phenomenon was observed in rats treated with ^{14}C aldicarb and ^{14}C aldicarb sulfoxide (Andrawes et al., 1967). Aldicarb was hydrolyzed faster than aldicarb sulfoxide in a 24 hour period, as determined by administering ^{14}C-carbonyl labeled compounds and measuring the evolved $^{14}CO_2$. The aldicarb hydrolysis products were difficult to isolate and detect; they may have been rapidly oxidized to the corresponding sulfoxides since the metabolites excreted in the urine were similar for both aldicarb and aldicarb sulfoxide treated animals. In the citron callus experiment the percentages of the sulfoxide present from application of aldicarb (67.3%) and of aldicarb sulfoxide (67.1%) were similar. In contrast to the above described data the citron data suggests rapid formation of aldicarb sulfoxide and very slow subsequent degradation of the sulfoxide once formed.

The percentage of aldicarb sulfone resulting from aldicarb application was generally low. The one exception was in the whole plant experiment where 12.4% and 7.4%

sulfone was detected in the foliage and stem, respectively. Similar percentages of sulfone were formed when sulfoxide was applied to the potato plants. Sulfone was not detected in the tuber callus and represented only 2.4% of the ^{14}C in citron callus at the last sampling period. As was discussed in Chapter III, the percentages of sulfone in the potato plant foliage and stem were relatively low when compared to another in vivo plant experiment using ^{14}C aldicarb in potato plants (Andrawes et al., 1971). It was postulated that the level of sulfone in the plant might be related to the level in the soil in which the plant was grown. It is known that soil composition (probably reflecting the microbial makeup) has an effect on the amount of sulfone resulting from aldicarb application (Smelt et al., 1978a, b and c; Bull et al., 1970; Bromilow et al., 1980). Therefore, the soil in which plants are grown could be an important factor in the quantitative aspects of various transformation products found in plants. Generally, thioether sulfur containing pesticides are metabolized to their corresponding sulfoxides but are metabolized to their sulfones to a much lesser extent (Kuhr and Dorough, 1976 and Hajjar and Hodgson, 1982). This is supported by the present data from the aldicarb callus tissue experiments where sulfone was formed only to a small extent. In addition, when sulfoxide was applied to potato tuber callus and citron callus tissues, again only small

percentages of sulfone were formed (1.0 and 3.2%, respectively).

When aldicarb sulfone was applied the lowest percentage of parent sulfone was found in tuber callus, 15.2%, compared to the 28.3% in tubers of in vivo potato plants. A much larger percentage of sulfone oxime was formed by tuber callus (42.7%) than the in vivo tubers (7.5%), while percentages of aqueous soluble ^{14}C were similar, 38.2% and 41.5%, respectively. In contrast, the sulfone was relatively stable in the foliage of whole potato plants and citron callus tissue, 64.2% and 68.2%, respectively. Other similarities between the potato plant foliage and citron callus were the percentages of aqueous radiocarbon, 16.1% each and percentages of unextractable residue, 2.9% and 2.8%, respectively.

Overall, there was good agreement in the qualitative distribution of metabolites resulting from application of aldicarb, aldicarb sulfoxide and aldicarb sulfone to whole potato plants and callus tissue cultures of potato tuber and citron fruit. The tissue culture systems gave a representative indication of the types of metabolites to expect in an in vivo experiment. Generally, however, the tuber callus degraded the parent compounds to a greater extent than did in vivo tubers. There were also higher percentages of conjugated species in the in vitro experiment. Feung et al. (1978) found that higher levels

of amino acid conjugates were formed from application of 2,4-D to soybean callus than in intact soybean plants and corn plants.

Mumma and Hamilton (1979) and Mumma and Davidonis (1983) have reviewed and compared experiments in which the metabolism of a number of pesticides have been performed in intact plants and in tissue cultures derived from those plants. They reported that there was generally good agreement in the metabolite patterns between the intact plants and tissue cultures. The primary differences were usually quantitative with higher rates of metabolism in the tissue culture.

The fate of xenobiotic chemicals in the environment should be assessed as long as they continue to be produced and used in such a way that their residues become dispersed in the environment. This is particularly imperative when pesticides are applied to agricultural food crops. In this situation, concerns arise about the potential human exposure to pesticide residues upon consumption of treated agricultural products. The Environmental Protection Agency requires extensive data on the behavior of a pesticide in the target crop as well as a battery of toxicological data before the pesticide can be made commercially available for use as an agricultural chemical. As a result, extensive information on the degradation and dissipation of

pesticides must be determined and a petition submitted to government regulatory agencies for registration.

Data used to compile registration reports, or reports on the behavior of pesticides in general, can be generated by a number of experimental methods. Ideally, the best data would be generated from experiments which would exactly replicate the environmental conditions under which the chemical would be administered. Obviously, this is not possible due to the various combinations of environmental parameters which can prevail. Therefore, at best, experiments can be performed to closely approximate the expected conditions. These would include in vivo experiments with intact plants grown under controlled conditions in growth chambers, plants grown in greenhouses and plants grown under actual field conditions. In vivo experiments would then inherently incorporate some aspects of external environmental factors (microbial degradation, photochemical reactions, etc) on the fate of the applied chemicals as well as whole plant effects involving penetration of the chemical into the plant and distribution within the plant. Although these types of studies yield information that somewhat reflect the "real" situation, they do not give accurate information on the actual metabolic capability of the plant. Further studies need to be performed to differentiate between specific metabolic pathways in plants and contributions from environmental factors. In vitro

methods can be used to accomplish this by using tissue culture techniques, isolated crude enzyme preparations or purified enzymes. Plant tissue cultures are ideal for this purpose, since they are grown under sterile conditions, can be grown in the dark and are intact cells containing all the necessary metabolic machinery. There is one possible major disadvantage in that cells in culture are less differentiated than native cells and may lose some metabolic capability, depending on the nutrient mix and hormonal constituents (Gressel, 1980) in their growth media.

APPENDIX

MASS SPECTRA

Figure A-1. Mass spectrum of aldicarb.

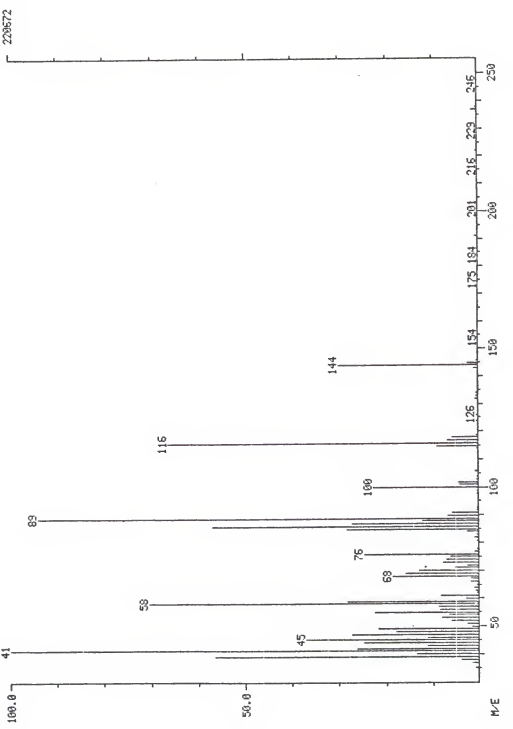

220572
100.0
50.0
M/E
41
45
53
68
75
83
100
116
144
50
100
126
150
154
175
184
200
201
216
229
245
250

Figure A-2. Mass spectrum of aldicarb sulfoxide.

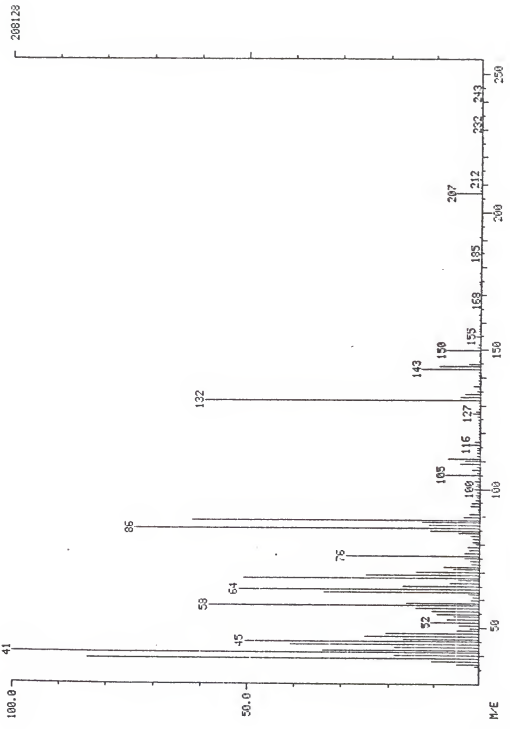

208128
100.0
50.0
M/E
41
45
52
59
64
75
86
105
116
127
132
143
150
153
168
185
207
212
232
243
250
200
150
100
50

Figure A-3. Reconstructed ion current for GC/MS of aldicarb sulfoxide nitrile.

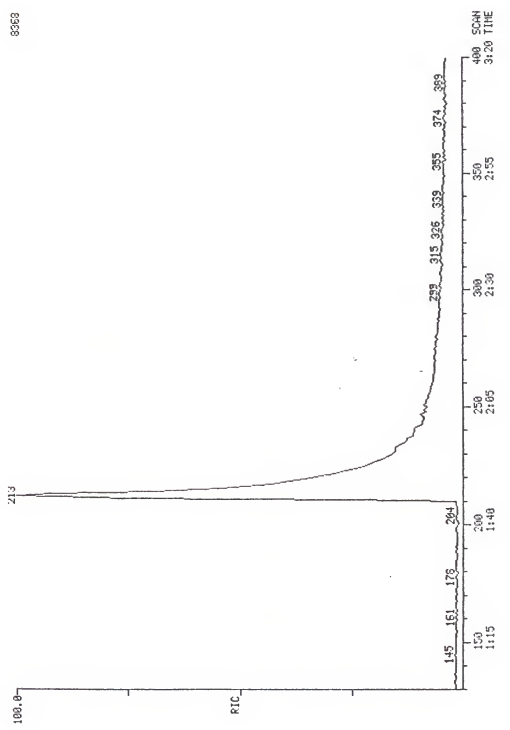

8363
100.0
RIC
213
145
151
173
204
150
1115
200
1140
250
2165
300
2130
350
2155
400
3120
SCAN
TIME

Figure A-4. Mass spectrum of aldicarb sulfoxide nitrile.

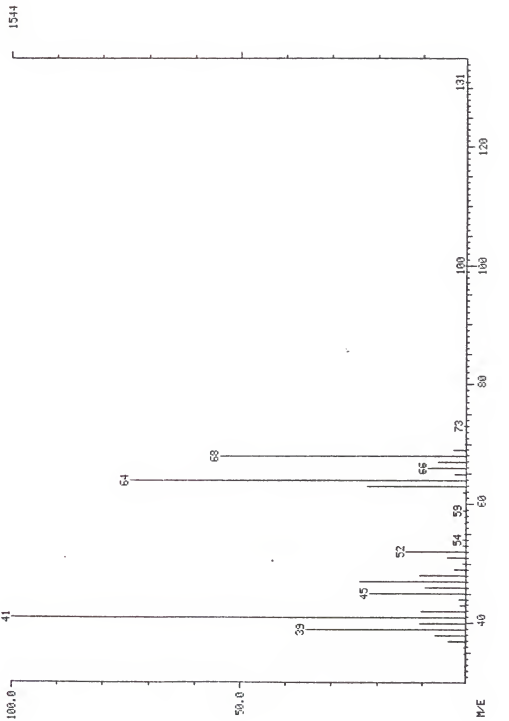

1544
100.0
50.0
M/E
41
39
45
52
54
59
64
68
66
73
131

Figure A-5. Mass spectrum of aldicarb sulfoxide oxime.

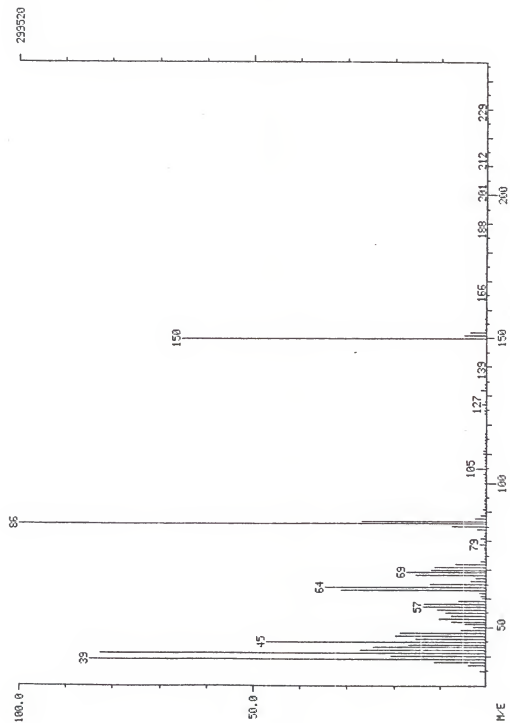

299520
100.0
50.0
M/E
39
45
50
57
64
69
73
79
86
105
127
139
150
166
188
201
212
229
260

Figure A-6. Mass spectrum of aldicarb sulfone.

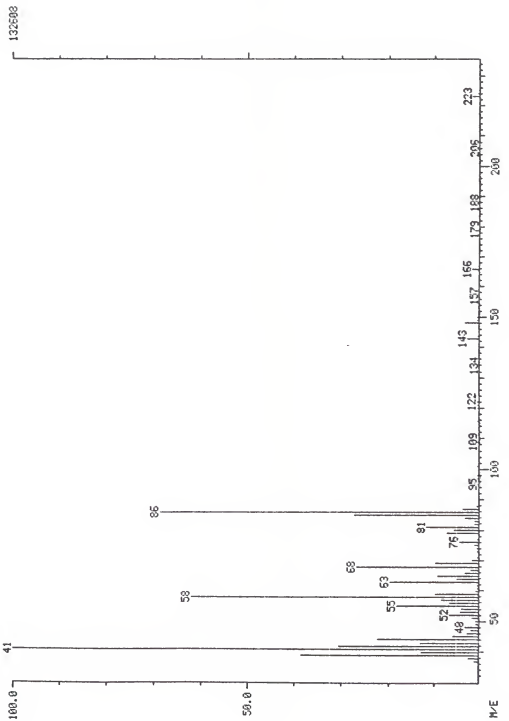

132608
100.0
50.0
M-E
41
50
52
53
55
63
68
76
81
85
95
100
109
122
134
143
150
157
166
179
188
200
206
223

Figure A-7. Mass spectrum of aldicarb sulfone nitrile.

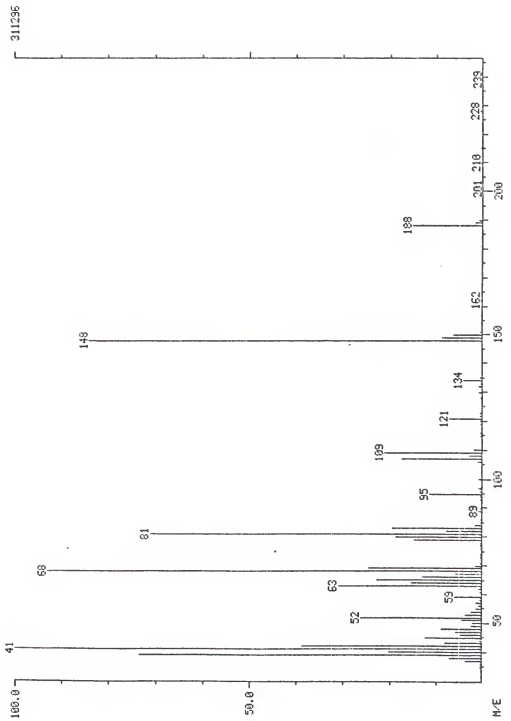
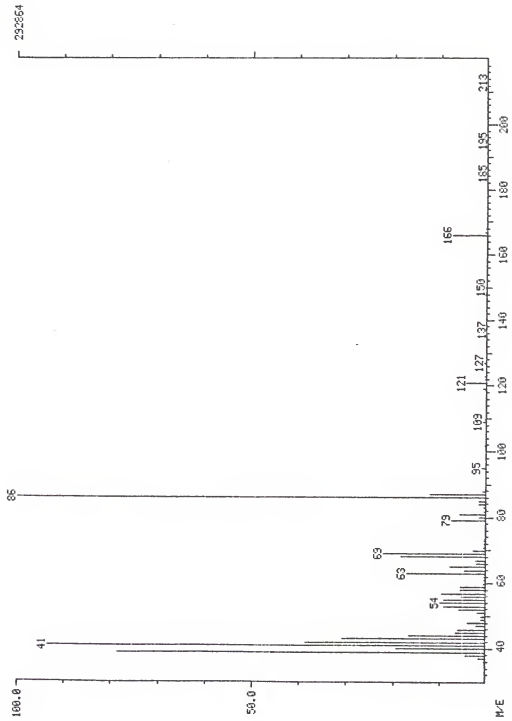

311295
100.0
50.0
M/E
41
52
59
63
68
81
89
95
100
109
121
134
148
150
162
188
200
210
220
229

Figure A-8. Mass spectrum of aldicarb sulfone oxime.


292564
100.0
50.0
M/E
41
54
63
69
79
86
95
103
121
127
137
166
40
60
80
100
120
140
160
180
195
200
213

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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